FOR OFFICIAL USE.

REPORTS

TO THE

LOCAL GOVERNMENT BOARD

ON

PUBLIC HEALTH AND MEDICAL SUBJECTS.

(NEW SERIES No. 76.)

Report to the Local Government Board upon the "Biological Properties" of Milk, both of the Human Species, and of Cows, considered in Special Relation to the Feeding of Infants. By Janet E. Lane-Claypon, M.D., D.Sc. (Lond.).

LONDON:
PUBLISHED BY HIS MAJESTY'S STATIONERY OFFICE.
To be purchased, either directly or through any Bookseller, from
WYMAN AND SONS, LIMITED, FETTER LANE, E.C., and
54, ST. MARY STREET, CARDIFF; or
H.M. STATIONERY OFFICE (SCOTTISH BRANCH),
23, FORTH STREET, EDINBURGH; or
E. PONSONBY, LIMITED, 116, GRAFTON STREET, DUBLIN;
or from the Agencies in the British Colonies and Dependencies,
the United States of America, the Continent of Europe and Abroad of
T. FISHER UNWIN, LONDON, W.C.

PRINTED BY
DARLING AND SON, LTD., BACON STREET, E.
1913.

Price Ninepence.

Report to the Local Government Board upon the "Biological Properties" of Milk, both of the Human Species, and of Cows, considered in Special Relation to the Feeding of Infants. By Janet E. Lane-Claypon, M.D., D.Sc. (Lond.).

ARTHUR NEWSHOLME,
Medical Officer,
4th January, 1913.

PART I.

INTRODUCTORY.

The great development which has occurred during the last quarter of a century in the various branches of Public Health, has been manifested to a high degree in the subject of Infant Feeding in relation to Infantile Mortality. The practice of boiling milk was introduced as a result of the discovery that the unboiled milk of a diseased animal when taken as food, could transmit disease.

In 1898, in connection with the fight against tuberculosis in Denmark, regulations were issued for the compulsory pasteurisation of milk, with a view to limiting the spread of this disease. In order to ensure that these regulations were carried out, it was essential to be able to test the milk, so as to detect any infringement of the regulations by the dairyman. For this purpose the property possessed by raw milk of giving colour reactions with certain substances, was utilised, this property being lost when the

---

* The small numbers in brackets throughout this Report refer to the Bibliography on pp. 81–94.

(25403—21.) Wt. 13093—129. 1000. 1/13. D & S.

milk had been heated to a definite temperature. These colour reactions are due to the presence of ferments, which are destroyed by different degrees of heat, and they will be dealt with fully in the next section. The mechanism of these reactions has been the subject of repeated research and of considerable controversy. The literature is voluminous, and much of it of but little value. The variations in the reactions as to colour with different reagents, the age of the milk, its cleanliness or dirtiness, and the precise temperature at which the reactions cease to be given, have all formed the subject of work, one investigator often rejecting the work of previous investigators, the general result being a bewildering mass of disputed details.

The realisation, towards the latter part of the 19th century, of the high rate of infant mortality which prevailed in most of the countries in Europe, led to a great increase in clinical observation on the feeding of infants.

Experience showed that those babies which received boiled cows' milk did not as a whole thrive so well as those which received raw mothers' milk, and it was first suggested by Escherich([61]) in 1900 that the cause of this difference might lie in the destruction of certain substances in the milk by the process of boiling. At that date very little was known about these substances, whatever they might be, and they were termed by Escherich "Stoffwechselfermente" or literally "Metabolism-ferments." The idea, once started, opened up a vast field for investigation, and numerous observers were not slow to enter it.

It was found that milk contains a number of ferments, and it was believed that the destruction of these by boiling might be responsible for the different results obtained with breast- and artificial-feeding respectively. Ehrlich([59]) and others had shown that passive immunity could be produced by suckling when the mother was immune; this led to the investigation of the presence in milk of precipitins, agglutinins, and other so-called "Protective substances" in milk.

These bodies, together with the ferments, form the group of so-called "Biological substances" in contradistinction to the more tangible chemical substances (sugar, protein, fats, salts and water) which constitute the directly nutritive part of milk.

One source of error permeates the greater part of the work upon the ferments of milk, namely, the simultaneous presence of bacteria, which produce ferments as a result of their activity. Such ferments differ in no appreciable point from those present in the organs of mammals, and might therefore be expected to occur, in a fluid, such as milk, derived from a gland.

The aim of this report is to investigate the presence of the biological substances; to discover how far they are due to bacterial contamination, and how far they are present in milk as such, apart from the contained bacteria; further, to endeavour to determine whether such substances can be considered to be of value to the infant, in aiding, or bringing about the digestion of the food-material of milk itself, or in producing immunity from the disease.

These biological substances will be dealt with in two groups—

1. The Ferments, and
2. The so-called "Protective Substances."

In dealing with the first group, for the sake of simplifying some of the difficulties, the literature and the original work carried out for the purpose of this report, will be taken together. Some of the work had already been carried out in conjunction with Dr. Harden before the commencement of this report, but for various reasons, the publication has been delayed, and the work, although carried out in 1911 has only recently appeared. The amount of original work required has been still further reduced owing to the recent appearance of several reliable papers (notably Rullmann's([149])), since the work for this report was commenced.

PART II.

ON THE PRESENCE OF FERMENTS IN MILK.

The history of the discovery of the ferments found to be present in milk, is intimately associated with the evolution of our present knowledge of the theory of ferment action.

The mechanism of the activity of ferment has been worked at both in plants and animals. It has been shown that all living cells contain ferments, and that although some of these ferments are highly specialised, and found only in one particular class of either animal or vegetable kingdom, yet there are certain main types of ferments which are found almost everywhere in nature.

At first, as the different reactions became known it was supposed that many of them were different manifestations of the same ferment action; but investigation of their thermal inactivation point, and of their distribution in nature showed that they were due to different ferments. This error has led to much confusion especially in the earlier literature, a confusion which has been further increased by the variety of terms which have been applied to the several reactions, owing very largely to the mechanism of their activity not having been understood.

At the present time, however, the nomenclature of the various reactions is fairly well established, and there is no apparent advantage in using any other terms than those now most generally recognised. Throughout this report, therefore, in dealing with the various ferments, only one name will be used for each ferment-action, even although the authors quoted may, some of them, have used another name.

The ferments which have been described as occurring in milk can be placed, roughly, under the following headings:—

1. Oxidising ferments.
2. Catalase.
3. Reducing ferments.
4. Proteolytic ferments.
5. Lipolytic ferments.
6. Lactase and glycolytic ferments.
7. Amylase.

In the following pages each ferment or group of ferments will be considered separately, and the progress of our knowledge concerning them will be traced down to the present day. Much of the literature is at the present time valueless, for it has been shown to be based upon investigations conducted without due consideration of the effects of bacterial contamination. Some authors have recently been able to eliminate this difficulty, and adopting similar methods, I have been able either to confirm the work of previous observers, or to test for the presence or absence of ferments not hitherto investigated upon these lines. At the same time these earlier papers, now chiefly of historical interest, have undoubtedly been stepping-stones to a clearer knowledge of the mode of action of these bodies.

On the clinical side it must, I think, be admitted that undue importance has frequently been attached to the ferments in milk, without sufficient consideration or investigation of their presence or possible origin.

It will be necessary to consider the ferments which have been found in the milk of different species; the most important milks for the present purpose are human and cows' milk, both of which will be dealt with fully, and the other milks briefly, and only in so far as their ferment content is known.

I.—The Oxidases.

The literature dealing with the presence in milk of oxidising ferments is very large, owing to several considerations. In the first place there has been considerable difference of opinion among investigators both as to the number of bodies which were to be classed among the oxidising ferments in milk, and further as to the specificity of these bodies. Secondly, the presence or absence of the reaction for one of those bodies has been extensively used as a test for the heating of milk, and since this was used in connection with legislative measures in Denmark, it was essential that the test should be reliable. There has again been much disagreement as to the most reliable form in which to apply the test for this body, and as to the mechanism of its action.

In order to simplify the somewhat complicated literature it was considered advisable to divide the question of the oxidising ferments into several sections, and to consider:—

1. The number of oxidising ferments stated to be present,
2. The tests employed for their identification,
3. The effect of heat upon the reaction used,
4. The mechanism of the reaction,
5. The presence of the peroxidase reaction in cows' milk, and
6. The presence of the reaction in human and other milks.

The literature dealing with the first four points will not be taken fully, only such work as is necessary for the essential facts being considered.

(1.)—*On the number of oxidising ferments stated to be present.*

Klebs (1868)([87]) seems to have been the first observer to discover the presence of an oxidising body in cows' milk. He showed that fresh milk coloured guaiacum tincture blue, that the same reaction could be obtained with both blood-protein and pus, and that in milk this reaction was connected with the protein. In the next year, Schaer([156]) (1869) pointed out that blueing of guaiac tincture could be obtained with certain salts, and that this reaction was one of oxidation, and also occurred if hydrogen peroxide were added. The process he believed to be one rather of ozonisation, and he showed that this body had the power of decomposing potassium-iodide-starch solution, with the liberation of iodine.

In 1881 Arnold([3]) showed that this reaction was destroyed in milk by heating to 80° C.

Babcock and Russell([8, 9]) (1889) found that the ferment solution prepared by them gave the reaction with guaicum, and they believed that it was another manifestation of the proteolytic activity of their solution (*cp.* p. 42), as was also the catalase reaction given by the same solution (*cp.* p. 15).

The separate identity of catalase and of the oxidising ferment was not recognised until some time later, and much of the literature even down to recent years shows confusion of thought and work between the two bodies. Others have, however, clearly recognised the difference between them, and among earlier authors Raudnitz([133]) (1898) stated his belief that the two reactions were due to two separate bodies.

Loew([105]) (1901) showed that solutions could be prepared from plants and other tissues which did not give both reactions, but only one of them, and that the reactions were therefore almost certainly due to different ferments. Further Neumann-Wender ([120, 121, 122]) (1903) showed that the thermal inactivation-point for the two reactions when present in the same solution, namely, milk, or in Babcock's ferment solution, were quite distinct. The separate identity and specific actions of the two were therefore established. It will be considered from this point onwards that the catalase and the oxidising reactions are separate, and attention will now be concentrated upon the latter.

Kowalewsky([96]) (1890) carrying on the work of earlier observers upon the action of milk towards guaiac tincture, showed that the reaction was most reliable when old guaiac tincture, which had stood in the light, was used, and Bourquelot([43]) (1897) showed that unless old tincture was used it was necessary to add hydrogen peroxide in order to obtain the reaction. Bourquelot believed that guaiacum must contain an aut-oxidisable substance, which became oxidised on standing. Following on these and other similar investigations it became recognised that the blueing of milk with guaiac tincture alone, was unreliable. This was thought to be explicable by the presence in milk of two oxidising ferments,

one of which was capable of acting with guaiac tincture alone, and was only irregularly present, and the other only capable of acting with the assistance of hydrogen peroxide. For these reasons the first of these bodies gradually came to be looked upon as a "direct" oxidising ferment or "direct oxidase" and the other as an "indirect oxidase" or "peroxidase."

Bach and Chodat([15, 16, 17, 18]) (1903 and after) in a series of papers have suggested that there are two ferments, the one which they call "oxygenase" and which from the context appears to be a peroxide, and not a ferment, and "peroxidase" which activates the peroxide or the "oxygenase," and gives rise to the blueing of the oxidisable substance. This suggestion appears to have been very largely accepted by subsequent writers.

Recently (1909) Moore and Whitley([117]) have shown that the oxygenase of Bach and Chodat is really a peroxide, and that when an apparent "direct oxidase" is found, it is due to the presence in the extract of a peroxide, so that the addition of hydrogen peroxide is unnecessary; when however the indirect oxidase action occurs, then there is no peroxide present, but it must be supplied by the addition of hydrogen peroxide.

Without considering the details of this most interesting work it will for the present be considered that there is only one ferment concerned in the production of the oxidase reactions, and the term "peroxidase" will be retained for this body. In dealing with the literature the question of the presence of an oxidase will be ignored, and the peroxidase reaction alone considered, whatever term may have been applied to the reaction by the author. The second point may now be considered, namely, the tests which have been employed for the identification of the presence of this body.

(2.)—*The Peroxidase Tests.*

In the years 1897 and 1898 the test for peroxidase in milk suddenly sprang into great importance. In France in the year 1897 an outbreak of foot-and-mouth disease occurred which was shown to be transmitted by the milk. An order therefore was issued for the boiling of all milk before it was sold, and in order to control the milk-purveyors it was necessary to have some reliable test which could be applied easily and rapidly to test the previous heating of the milk sold. Bourquelot([43]) in working upon the test with this object used guaiacum tincture and found that it was necessary to add hydrogen peroxide if the reaction was to be reliable.

In 1898, with the object of controlling bovine tuberculosis, it was enacted in Denmark that all milk must be pasteurised before it was sold. Storch was appointed to work out a reliable test which could be used to differentiate raw from heated milk. Storch([183]) tried several reagents, and finally decided in favour of para-phenylene-di-amine with the addition of hydrogen peroxide. This test is known as Storch's test, and when carried

out in fresh milk gives deep blue-grey colour almost instantly, on the addition of the reagent. In boiled milk, however, the reaction is negative or delayed, and Storch believed that this test was quite reliable for differentiating between milk which has been heated to 80° C. and over, and milk which had not been heated to so high a temperature.

It was inevitable that considerable discussion should arise about so important a reaction dealing with public health matters, and the literature upon the details of the peroxidase reaction is voluminous. Many observers still preferred the guaiac reaction even after Storch's test was published, and they endeavoured to find some means which would render the reaction reliable. Some, like Kowalewsky and Bourquelot, believed that the use of old tincture was the best plan; others preferred to use guaiacum bark and others the guaiacum resin; Seigfeld ([170, 171]) advocated the use of an acetone solution of guaiacum, which he believed to be always reliable.

Much discussion was aroused by the introduction of Ursol D. as a reagent (*cp.* Arnold and Menzel([4]), Glage([69]) and v. Itallie([82]), Weber([203]), Wirthle([206]), Zink([209]), Chlopin([63]) and Utz([189])) in the place of para-phenylene-di-amine; many other substances which strike a colour on being oxidised have been suggested and to some extent used.

As a whole it seems that the guaiac reaction and Storch's reaction have been the most widely used, Kastle and Porch([86]) (1908) found that Storch's reaction could be sensitised by the addition of a one per cent. solution of trikresol, and Rothenfusser (1908 and 1910) found para-phenylene-di-amine-hydrochloride more reliable than the base itself. Rothenfusser([144, 145]) further suggested a mixture of guaiacol and para-phenylene-di-amine as a reagent. This mixture strikes a brillant violet with raw milk, and is known as Rothenfusser's reagent. The serum obtained after treating milk with lead acetate is recommended by the author to be used for the test. Before dealing with the cause of this reaction the effect of temperature upon the peroxidase reaction in milk must be considered.

(3.)—*The Effect of Heat upon the Peroxidase Reaction.*

In using this reaction for the purpose of detecting the previous heating of milk it is evidently necessary to know at what temperature this reaction ceases to be given. The numerous observations upon this point are by no means concordant. It is not possible to deal in detail with the various results obtained, but the accompanying table, compiled in great measure from the tables given by Waentig([200]) (1907) and van Eck([58]) (1911), will show the most essential points. It shows the great difficulty of deciding upon the precise temperature at which the reaction ceases to be given, and therefore the exact temperature to which the milk may be considered to have been heated.

Table showing the Inactivation-point for Peroxidase, after different Authors.

Author.	Reaction persists at	Is destroyed by temperature of
Dupouy[56]	... ...	80° C.
Storch[183]	75° C. for 2 minutes ...	79–80° C.
Leffmann[100] ...	76·5° C. (still active) ...	82° C.
Tjaden, Koske, Hartel[185]	Below 80° C.	Over 90° C. Depends upon time.
Rullmann[146, 147] ...	½ hour at 70° C. (weak after 5 minutes).	75° C. for 10 minutes or 69–70° C. for 1 hour.
Schweitzer[162] ...	About 1 hour at 65° C. ...	
Utz[189, 190]	71° C. for 1¼ hours ...	
Neumann-Wender[120]	... ...	83° C.
Seligmann[168] ...	... ...	72° C. for 15 minutes.
	... ...	75° C. for 5 minutes.
	... ...	76° C. for 1 minute.
Butterberg[49] ...	... ...	70° C. for 30 minutes.
Koning[88]	86° C. if heated quickly ...	73–74° C. if heated slowly.
Kastel and Porch[86]	... ...	70° C. for 1 hour.
	... ...	75° C. for 20 minutes.
Giffhorn[67]	... ...	72° C. for 30 minutes.
Van Eck[58]	Depends upon time of heating.	

Van Eck (1911) arranged a series of test tubes containing varying amounts of raw sterilised milk. (The sterilised milk was heated to 100° C. for ½ hour.) The series ranged from 9·9 cc. sterilised milk and ·1 cc. raw, to 8 cc. raw and 2 cc. sterilised. After adding equal amounts of the reagent (Storch's test was used) these tubes formed a scale of colour which was used for each experiment as a standard colour scale, the colour depending upon the amount of fresh milk, that is upon the amount of ferment present in each tube.

He then took some of the same milk and heated it gradually in a water-bath fitted with a stirrer, and, taking samples at different temperatures and intervals, he compared them with the standard colour scale for that experiment.

Using the values thus obtained he found that the reaction followed the law for monomolecular reactions, and that the equation $K = \frac{1}{T} \log_e \frac{a}{a-x}$ (where K = a constant, and a = concentration at time O, x = concentration at time T), was true, and that hence the reaction was monomolecular in type. The effect evidently depends upon the length of time over which the heat is maintained, and van Eck points out that it is impossible with any one test to discover both the temperature and the length of time of heating.

There is no means of testing the amount of peroxidase present in milk; van Eck's work deals with the disappearance of such amounts of peroxidase as are present in the particular sample of milk considered, and not with absolute quantities. Further, the test for peroxidase can readily be restored to heated milk by the addition of a small amount of raw milk, if the presence of the reaction is required.

(4.)—*The Mechanism of the Peroxidase Reaction.*

The oxidising ferments have always attracted much interest among scientific workers, but, although a large amount of work has been done upon them, it cannot be said that their action is fully understood.

It appears that at any rate one cause for the production of the peroxidase reaction is due to the presence of either iron or manganese, probably in colloidal form, since a metal sol will also give the reaction.

Bertrand([34, 35]) (1897) showed that the oxidising ferment which produces the Japanese lacquer acts only when manganese is present, and Sarthou([152]) (1900) found that another oxidising ferment obtained from a plant owed its activity to the presence of iron.

Engler and Wohler([60]) (1904) suggested a theory whereby the action of the ferments in milk are brought into line with the action of a metallic sol. This suggestion was carried further by Bordas and Touplain([40, 41]) (1909). They showed that the ferment reactions of milk are given by colloidal solutions of metals, and that these same reactions can also be given by solutions of lactate and oxalate of iron. They also showed that milk, if heated to 85° C. and then pulverised by being projected in a fine jet under high pressure on to an agate plate, would again give the reactions for peroxidase and catalase, and they believed this to be due to the physical condition of the milk. They found that milk could also be re-activated by pumice. J. Meyer([110]) (1910) confirmed the findings of previous observers and showed that boiled milk could be re-activated by the addition of pumice, kaolin, or better still, platinsol.

Moore and Whitley([117]) found iron and manganese constantly present in the peroxidase solution made by them from potato juice; the only observer who failed to find either one or other metal in such a solution was (as far as I can ascertain) Bach([18]) (1910), who, however, gives no details of the method used nor of the degree of accuracy of his tests.

Van der Haar([74]) (1910) prepared an active peroxidase solution from the leaves of Hedera Helix and from potato juice. The solution always contained manganese in varying proportions (from ·00023 per cent. to ·03 per cent.), the amount not varying directly with the strength of the ferment reaction.

Grimmer([71]) (1911 and 1912) prepared a peroxidase solution from milk. The ash of this solution was found to always contain iron and frequently manganese, this last in very small amounts.

Sarthou([154]) (1911) showed that the peroxidase reaction may be brought about by infinitesimally small amounts of colloidal iron or manganese, the action of the latter being assisted by the presence of iron; a solution containing ·0002 per cent. of iron is sufficiently strong to bring about the reaction.

It has also been shown by several observers that the guaiac reaction can be given by other salts, especially chlorides. Thus, Alsberg([2]) (1908), working with the blood of sea-water animals, showed that sodium and potassium chlorides acted as sensitisers

for the peroxidase reaction, and that the intensity of the action varied with the concentration of the salt. He believed the guaiac test to be entirely unreliable. Sarthou([154]) and Sartory([155]) (1911) both pointed out the same facts. Sartory worked with the water of Breuil and showed that this mineral water even after being bottled for two years will still give the peroxidase reaction with guaiac and hydrogen peroxide, and has catalytic properties.

There is no difficulty in supposing that the salts of iron and possibly manganese are concerned in the peroxidase reaction in milk. It has been shown that Grimmer found these last two metals in the solution made by him from milk, and iron is well known to be constantly present in both human and cows' milk, but I am unaware of any evidence as to the condition of the metal, whether colloidal or not. The most recent work upon the iron in milk is that of Langstein([99]) (1911), in whose paper further references can be found. Several authors have shown that the peroxidase reaction depends upon the alkalinity of the milk. Thus Kooper([91]) (1910) and Hesse and Kooper([93]) (1911) showed that as the acidity of milk increased the peroxidase reaction became weaker. They worked with Rothenfusser's reagent and showed that the alkalinity of the milk decreases on boiling and the reaction disappears. If, however, a little alkali is now added the reaction at once returns. This is, however, not the case if the alkali is added before boiling; in this case the alkali appears to be bound, for the total alkalinity is decreased, and the reaction is not given.

Grimmer([71]) (1911 and 1912) did not entirely agree with Hesse and Kooper, for he found that if the milk is heated above 100° C. the addition of ammonia did not restore the reaction. He prepared a solution by dissolving the precipitate obtained by the complete saturation of whey with ammonium sulphate and found that this gave a strong peroxidase reaction. He concludes that the ferment must either be of the nature of lact-albumin or that it must be absorbed by the protein. The reaction was abolished if the alkalinity of the solution was raised to that of a $\frac{N}{40}$ soda solution. The ferment action was also entirely destroyed by digestion with either pepsin or trypsin.

One more point remains for consideration which has been already mentioned on p. 6, namely, that of the relation of the peroxidase to a peroxide. Neumann-Wender([120]) (1903) found that active guaiac tincture, that is, one giving the "direct" oxidase reaction, was inactivate by boiling, and he believed that the active tincture contained a peroxide. That this is the case was definitely shown by the work of both Arnost([5]) (1905) and Waentig([201]) (1907). Guaiacum contains a substance which becomes oxidised on exposure to the air, and forms a peroxide. If this substance is formed in sufficient quantity the tincture is "active," and the peroxidase reaction is given without the addition of hydrogen peroxide. When guaiacum is digested with acetone, as was done by Siegfeld in making his active solution, this substance is formed.

The work of Bach and Chodat has been mentioned already on p. 6. From their work and from that of Moore and Whitley([117])

(1909) it appears that a peroxide is present in many of the plant juices. Moore and Whitley worked with vegetable extracts and also with milk. An active plant juice which requires no addition of peroxide when quite fresh becomes inactive on standing or warming to 50-60° C., the reaction being then only given when hydrogen peroxide is added. They prepared a solution of peroxidase from the juice of potatoes and found that the solution was colloidal in character, reduced Fehling solution, did not give the protein tests, and was completely destroyed by an acid or by alkali in more than the merest trace.

The peroxidase reaction seems, therefore, to be dependent for its production upon the reaction of the medium, and probably upon the presence of a metal in colloidal condition. The reaction can also be brought about by certain salts.

(5.)—*On the Presence of the Peroxidase Reaction in Cows' Milk.*

It has already been stated that no observer has failed to find the reaction for peroxidase present in cows' milk, and it only remains therefore to consider the possibility of the reaction being due to bacteria, and the portion of the milk to which the body giving this reaction is attached.

As regards the first point it has been shown by numerous observers, apart from the evidence already given in the previous section, that the reaction disappears as the alkalinity of the milk becomes lessened; this is somewhat against its bacterial origin although not entirely, since it is well known that the bacteria themselves die as a result of the increasing acidity of the milk brought about by their own activity. The only observer who appears to have obtained a positive result with bacteria is Bellei([31]) (1904), who found the peroxidase reaction positive in milk previously boiled and then inoculated with B. Pyocyaneus, though the reaction was slower than in fresh milk. Much stress cannot be laid upon this observation, which has not been confirmed by any subsequent observer.

Jensen([83]) (1906) tried to detect the peroxidase reaction in bacterial cultures of the commoner milk organisms, including some of the lactic-acid-forming varieties, the results being in all cases negative. Oidium Lactis and Penicillium Glaucum showed a blue colour inside the hyphae, but not outside, thus showing that the ferment was present inside the cell of the plant. He thinks, therefore, that the peroxidase reaction in milk is not due to bacteria.

Barthel([23]) (1907) also showed that milk rendered sterile by chloroform and toluol gives a positive reaction for peroxidase.

Rullmann([149]) (1911) working with initially sterile cows' milk found the peroxidase reaction always positive, and Harden and Lane-Claypon([74]) (1912) working with the same class of milk obtained identical results.

It appears therefore that the peroxidase reaction is not due to bacterial contamination.

As regards the part of the milk concerned with the production of this reaction, the evidence already given shows conclusively that it is concerned either with the lactalbumin or with some similar body.

Klebs([87]) (1868) as already mentioned (*see* p. 5), believed it to be due to the caseinogen, whilst Babcock and Russell([9]) (1898) found that the slime obtained on centrifuging milk gave a very strong reaction.

Kowalewsky([96]) (1890) believed that the reaction was connected with the lactalbumin, and Raudnitz([133]) (1898) found that the active substance was precipitated with different fractions according to the precipitant used. Later (1903) he considered that it came down with the caseinogen, but was not attached to it.

Barthel([21]) (1899) thought that the reaction was dependent upon the caseinogen as also did Bordas and Touplain([40, 41]), who considered that the whole reaction was a function of the caseinogenate of calcium and not of a ferment at all. They found also that both the cream and sediment of boiled milk gave the reaction. Monvoisin([112]) (1908) found the reaction positive in the filtrate obtained after saturation with magnesium sulphate, that is after the caseinogen had been removed; hence it is not dependent upon the globulin fraction.

Meyer([110]) (1910) criticising Bordas and Touplain's work showed that caseinogen alone, whether prepared by 24 hours' incubation, by the addition of tri-chlor-acetic acid, or by rennet, from either raw or boiled milk did not give the peroxidase reaction. He obtained a positive reaction with the cream from centrifuged milk as well as with the fluid, and a weak effect with the sediment.

Kooper([91]) (1910) found that the peroxidase remained behind in the fluid on centrifuging, and did not go up with the cream; and Sames([150]) in the same year showed that it was probably attached to the albumin molecule and that the reaction was strongest in the first milk and often absent in the strippings.

If to these results the work of Grimmer recorded in the previous section (*see* p. 10) be added it seems fairly evident that at any rate the major part of the substance bringing about the peroxidase reaction must be associated with the albumen fraction. Weber([204]) (1910) found " oxidase " present in colostrum, except sometimes on the first day after birth. Giffhorn([67]) (1911) found the reaction in cows' milk much stronger in cases of mastitis, than in normal milk.

Grimmer([70]) (1910) in his work upon the mammary glands of various animals found that the glycerine extract, made without injury to the cells, was invariably negative to the peroxidase test, except in one case in which there was mastitis. If, however, the glands were ground up with quartz, then the result was in all cases positive, whether the gland was lactating or not, even in the case of the mare and sow, whose milk did not give the peroxidase reaction (*see* p. 14).

The results of sections (5) and (6) will be summarised together.

(6.)—*On the presence of the Peroxidase Reaction in Human Milk.*

Raudnitz (1898) failed to obtain the peroxidase reaction with human milk using guaiacum and hydrogen peroxide; and showed that this was not due to any inhibitory body, since when human milk was added to cows' milk the reaction with the cows' milk took place just as easily. He found, however, that human colostrum was active, and that the active substance was precipitated with the globulin fraction. It may be pointed that Raudnitz found the same for cows' milk, in respect of this last point.

Moro([113]) (1902) using the oxidation of salicylic aldehyde as a measure of the oxidising power, obtained negative results with human milk.

Spolverini([180, 181]) (1902 and 1904) found a weak reaction with human milk, and showed that it became stronger if the milk assumed the character of colostrum. He believed that the ferment was attached to the leucocytes.

Nordmann([127]) (1902) published a case of a child who was fed upon the breast of a mother who was suffering from mastitis. The infant did not thrive, and on testing the milk it was found to be negative to Storch's reagent. Three samples of milk taken from other women gave a positive reaction, and Nordmann apparently attributed the infant's lack of progress to the absence of peroxidase. This paper produced a reply from Thiemich([184]) (1903), who showed that the presence of peroxidase is altogether uncertain. He examined the milk of a large number of women attending the Breslauer Klinik, and found that whilst it was usually present it was very inconstant.

Marfan and Gillet([109]) (1902) found that the reaction is present in colostrum, but disappears as the gland gets into full work; if, however, the gland is allowed to become less active, then the peroxidase again appears, and with it the leucocytes; the peroxidase reaction may, however, appear rather earlier than the leucocytes. They further found that if milk giving the peroxidase reaction be examined under the microscope, on the addition of guaiacum and hydrogen peroxide, the area around the nucleus of the polymorpho-nuclear leucocytes became blue. The fluid around also showed a faint bluish tinge, so that, apparently, the substance can pass out.

Gillet([83]) carrying this work further, showed that the presence of peroxidase is due to leucocytes, and that it is found both in the fluid and in the sediment of centrifuged milk.

He also examined the milk from a large number of women, and found, as Thiemich did, that the reaction was extremely uncertain; it varied in the milk from the two breasts.

Friedjung and Hecht([64]) (1903) working on the milk from a large number of women, found that the reaction was negative in 114 out of 174 samples of milk examined, and varied greatly in intensity. As a whole the reaction was more marked in colostral milk, but it was often absent even here.

Jolles([84]) (1904) was so entirely unable to detect the presence of peroxidase in human milk that he suggested that the test should be used as a means of distinguishing cows' milk from human milk.

Kastle and Porch([86]) (1908) found the results of the peroxidase reaction very uncertain with human milk, even with the addition of their sensitiser trikresol; the colostral stage appeared to have, as a whole, greater activity than the later milk.

The peroxidase reaction has been found positive in goats' milk, by Spolverini([179]) (1902), Luzzati and Biolchini([38]) (1902), Raudnitz([133]) (1898), Grimmer([70]) (1910), Harden and Lane-Claypon([75]) (1912), and by Weber([204]) (1910), who worked with colostrum only.

It was positive in sheeps' milk. (*Cp.* Raudnitz, Grimmer, and Weber.)

Raudnitz (1898) found the reaction negative in the ordinary milk of the horse, dog, ass, and rabbit, but positive in the colostrum of these animals.

Spolverini (1902) found it weakly positive in the milk of dogs and asses, and Grimmer found it negative in the milk of the horse and pig.

Reviewing the work described in sections 5 and 6, it appears that peroxidase is constantly present in the milk of cows, and only inconstantly present in human milk. There is no reason to suppose that it is bacterial in origin.

Comparing the work of Weber, Giffhorn, Grimmer, Spolverini, and Marfan and Gillet, it appears that peroxidase is derived from cellular elements, and at any rate in great part, from the leucocytes.

2.—Catalase.

The name catalase is now very widely, if not universally, applied to the ferment, generally found in both animal and vegetable tissues, having the property of splitting hydrogen peroxide into water and oxygen, with the formation of molecular or inactive oxygen, according to the equation—

$$2H_2O_2 = 2H_2O + O_2.$$

The name of this ferment has been the subject of considerable discussion, and it has not infrequently been called superoxydase; in this report, however, the more generally accepted nomenclature will be followed throughout, and the ferment will be termed catalase, even in dealing with papers by authors who prefer the other term.

The literature dealing with the presence of this body in the milk of various animals is extremely voluminous, and much of it does not present sufficient interest for it to be necessary to deal with it in detail. Many of the papers are concerned with the attempt to find a relationship between the catalase content and the purity or otherwise of the milk; this aspect will be referred to somewhat briefly, as it does not come strictly within the scope of the present investigation.

On the Presence of Catalase in Cows' Milk.

The existence of this ferment was known for many years before its presence in milk was detected.

In 1882 ([27, 28, 29]), Bécamp and Bert and Regnard([33]), showed that this property was common to extracts of practically all the

tissues they examined. Bécamp showed that fibrin was very rich in this substance, and that it could split many times its own volume of hydrogen peroxide.

Babcock([7]) (1889) discovered that milk decomposed hydrogen peroxide in varying quantities and, apparently owing to the work of Bécamp, concluded that fibrin must be present in milk, although he was quite unable to demonstrate its presence. Babcock also found that the strippings were the most active parts of the milk in this reaction, but he did not think that there was any definite relationship between the intensity of the reaction and the fat content, since the action persisted even after the cream had been removed. Colostrum was from 10 to 15 times as active as ordinary milk.

Babcock pointed out that in estimating the amount of gas evolved by the action of the ferment, it was necessary to shake constantly in order to obtain accurate results.

Later, Babcock working with Russell([8, 9]) on the ripening of cheese, prepared a ferment solution from milk which had proteolytic activities, and also had the power of splitting hydrogen peroxide. They concluded that this was another aspect of the activity of the proteolytic ferment; later again, they found that this solution also gave the peroxidase reaction, and considered that this last reaction could be taken as a measure of the catalytic activity of the solution.

Raudnitz([133]) (1898) found catalase present in cows' milk, and considered that it must be different from peroxidase because it was present in human milk (*cp.* p. 13). Also he showed that peroxidase and catalase had different reactions towards precipitating agents.

Lépinois([103]) (1899) came to the same conclusions as Raudnitz, namely, that the different reactions are due to two ferments, because in solutions of tissue extracts which gave both the catalase and the peroxidase reactions, (*a*) heating to 70° C., destroyed the power of the solution to split hydrogen peroxide, but did not affect the oxidising power, and (*b*) in fresh extracts if alcohol be used as a precipitant it is possible to obtain a precipitate which has catalysing but not oxidising powers.

Storch([183]) (1899), who apparently did not consider that the reactions were due to separate bodies, was able to prepare a solution of lactalbumin, which had been dried at 40° C. and showed catalytic but not oxidising power.

Barthel([21]) (1899) considered that the catalytic action described by Babcock and Russell was due to the leucocytes, which were not, however, the only constituents of the blood to possess this property.

In 1901 Loew([105]) published his well-known paper on catalase, which, although it does not deal directly with the catalase of milk, contains many points which are of sufficient importance for it to be necessary to deal briefly with his work. He suggested the term catalase for this ferment, and subsequently upheld this term in preference to " superoxydase."

Loew showed that the power of splitting hydrogen peroxide must be distinct from that of oxidising, since extracts could be pre-

pared which showed the presence of catalase and not of peroxidase and *vice versa*.

Dealing with the occurrence of catalase, he says:—

> "Numerous tests have established beyond a doubt that catalase is of general occurrence in the vegetable kingdom. No living plant or vegetable organ tested was found free from it." And also, "In the animal kingdom catalase is also of universal occurrence. The aqueous extracts of spleen, pancreas, liver, kidney, brain, muscle and blood serum show the power of catalysing hydrogen peroxide."

Loew also found that bacteria were capable of producing catalase, notably B. Pyocyaneus. In estimating the amount of oxygen evolved by catalase, he emphasises the importance of continuous shaking.

In a further paper, Loew deals with the probable importance of catalase in the tissue extracts. He believes its presence to be essential for the removal of hydrogen peroxide, which appears to be present in the living cell, and yet to be harmful to it. In this view he differed from Bach and Chodat, who considered that hydrogen peroxide was not harmful to living tissue.

Chick([52]) (1901) worked upon the presence of catalase in milk, in the course of some work undertaken in order to test the value of hydrogen peroxide as a preservative for milk. This method of sterilising milk had recently been suggested by Buddé, and the process is, therefore, known as Buddisation. She showed that milk when raw had the power of splitting hydrogen peroxide, but that this power was lost upon the addition of fairly large quantities of the reagent, when sterilisation of the milk was obtained. If, however, milk which had been sterilised by boiling was inoculated with a little raw milk, the power of splitting hydrogen peroxide, which had been destroyed by the boiling, gradually returned. Both these facts pointed to the production of catalase by bacteria, but it did not at all follow that the catalase was produced by bacteria alone.

Later (1903), Renard([140]) found that the catalytic power of milk was inhibited by the addition of 3 per cent. H_2O_2, and that milk which had been heated to 75° C. could be very well preserved by this means.

Some observers about this period had believed that catalase was a reducing ferment, but Bach and Chodat (1903) showed that it was a specific substance whose action was to split hydrogen peroxide, and that only. They prepared substituted peroxides, and found that the ferment did not attack them. They also showed that catalase as such has no reducing power, and that it is quite distinct from oxidising ferments.

This view was further confirmed by Neumann-Wender (1903), who prepared a solution of ferments from milk, after the method of Babcock and Russell, and showed that—

(*a*) the proteolytic power was destroyed by heating to 75° C.
(*b*) ,, catalytic ,, ,, ,, 80° C.
(*c*) ,, oxidising ,, ,, ,, 83° C.

He concludes that the ferment solution of Babcock and Russell was, therefore, composed of a complex of ferments.

From this date it seems to have been fairly well established that catalase is a specific ferment, and it will be dealt with as such in this report.

The consideration of this ferment can most conveniently be divided into several sections:—

1. The source of the catalase in cows' milk.
2. The estimation of catalase.
3. The relation of the catalase content to the condition of the milk.

Section I.—The Source of the Catalase in Cows' Milk.

There appears to be a special affinity between the catalase and the fat content of the milk, since if the milk be centrifuged the major part of the catalase goes up with the cream. The catalase does not, however, appear to be directly attached to the fat globules, since it can be washed away fairly easily. The total amount of catalase present is very variable, as was pointed out by Raudnitz[136] (1903), even when the conditions were kept constant as far as was possible.

Reiss[139] (1905) showed that catalase can be dissolved out from the cream, obtained by centrifuging, by either water or saline, and that the fat globules are not injured. This work was confirmed in the next year by Seligmann, who gave the following figures:—

25 c.c. skim milk gave	1·2 c.c. O_2	with	0·5 c.c. perhydrol	in	1 hour	at	37°	C.
„ whole milk „	4·0 c.c.	„	„	„	„	„	„	
„ cream „	7·3 c.c.	„	„	„	„	„	„	
Watery extract from cream „	12·0 c.c.	„	„	„	„	„	„	

Jensen[83] (1906) estimated the oxygen evolved from the different portions of milk, first milk, middle milk, and strippings, and found a well-marked connection between the fat and the catalase content (*see* table on p. 18). He showed that the strippings contained more leucocytes than the rest of the milk, and believed that small quantities of the catalase was derived from the leucocytes, the greater part being bacterial in origin.

Heygendorff and Meurer[78] (1910) also found more catalase in cream than in the rest of the milk. In the same year Meyer[110] showed that catalase is present in the cream, the skim milk, and in the sediment, in the last only in small quantities, unless dirt be present, when it is increased. This was also found by Harden and Lane-Claypon (*see* p. 19), and other observers have obtained similar results.

This does not touch the question of the origin of the catalase, which must now be considered. Chick's work, already quoted, showed that bacteria could produce catalase in milk, and this has been shown by a number of subsequent observers.

Seligmann[164, 165, 166] (1905 and 1906) believed that the catalysing and reducing powers of milk were closely connected, and that both were due to bacteria alone. He isolated a strain of cocci from milk, which, on being cultured, gave large quantities of catalase, and considered these to be the source of the catalase in milk.

Koning([88]) (1906) found the catalase content very variable, and showed that bacteria were able to produce catalase but not peroxidase, and hence concluded that these were two different bodies.

Jensen([83]) (1906) showed that many bacteria will produce catalase, among others Staphylococcus Aureus, Proteus Vulgaris, and P. Zopfii, B. Prodigious, and Oidium Lactis.

He collected milk into sterile tubes with all precautions against bacterial contamination, and estimated the fat and bacterial content, and the oxygen evolved from 10 cc. milk.

The figures given on the following table show that there is a marked connection between the fat and the catalase, but not between the small number of bacteria and the catalase.

—	Fat Content. Per cent.	Bacteria per c.c.	Oxygen from 10 c.c. Milk.
Cow 1:			
First milk	·55	160,000	Trace.
Middle milk	2·70	480	·5 c.c.
Strippings	8·30	360	2·0 c.c.
Cow 2:			
First milk	1·5	3,200	·5 c c.
Middle milk	3·4	2,800	1·0 c.c.
Strippings	7·8	360	1·5 c.c.

Sarthou([153]) (1909 and 1910) writing against Bordas and Touplain (*see* p. 19) showed that if milk is carefully collected it contains very little catalase immediately after milking, but that on keeping the quantity increases, or, if after heating, the milk is inoculated with a lactic-acid-forming organism.

Kooper([91, 95]) (1910 and 1911) showed that the catalase content of milk was greater on the second day after milking than it was on the first, and believed this to be due to the development of the bacteria. If milk was collected with all possible precautions against bacterial contamination, the amount of catalase was much less, but that the content in this milk was greatly increased by the addition of a small piece of solid excreta. He also showed that boiled milk can be re-activated by inoculation with raw milk.

Faitelowitz([61]) (1910) agreed with previous observers as to the increases of catalase in milk on keeping. The addition of chloroform or formalin inhibited the increase of catalase, which he believed to be due to bacteria.

There can be little doubt that the greater part of the catalase in milk is bacterial in origin, but it is not yet apparent from the work already given that this is the sole source of the catalase. Barthel([24]) and Smidt([173]) (1908) working independently both showed that milk which has been rendered sterile by the addition of antiseptics gives the catalase reaction, and believed this to indicate that there was a source of catalase other than bacteria.

This missing link was supplied in 1911 by the work of Rullmann, and has been confirmed by Harden and Lane-Claypon in work undertaken before Rullmann's results were published, but only actually in process of publication at the present time.

Both Rullmann and ourselves obtained the milk by means of the milking-tube; milk thus obtained giving a negative bacterial count.

In 20 samples Rullmann obtained complete sterility, and in others the bacterial count was negligible. He used 20 cc. of milk, and 10 drops of 3 per cent. H_2O_2. Koning's method was employed, and the samples were kept on ice. Summarising his results the sterile samples gave the following O_2 figures:—

After 1 hour—Nil, or only a trace.
After 12-18 hours—0·2-3·8 cc.
After 18-24 hours—0·2-3·6 cc.

In milk which was not entirely germ-free rather more oxygen was evolved after the longer periods of observation than in the case of the sterile samples. Dr. Harden and Lane-Claypon([75]) found catalase present in all cases, but the amount of oxygen given off varied within wide limits. The following table gives the summary of the results of the more important experiments:—

Material.	Bacterial Count. Per c.c.	Oxygen Evolved.	Time Allowed.
Exp. 1:			
50 c.c. whole milk	Nil	3·7 c.c.	3 hrs. 50 min.
Exp. 2:			
50 c.c. whole milk	47	6·0 c.c.	3 hrs. 25 min.
Exp. 3:			
50 c.c. whole milk	2·0	12·6	4 hrs.
45 c.c. skim milk	2·0	2·8	4 hrs.
Cream from 45 c.c. milk (the cream was made up to 45 c.c. with sterilised saline).	2·0	4·5	4 hrs.
Exp. 4:			
50 c.c. whole milk	6·0? plates spoiled.	5·9 c.c.	50 mins.
45 c.c. skim milk (no sediment) ...	Ibid.	2·4 c.c.	50 mins.
Cream from 45 c.c.	Ibid.	0·9 c.c.	50 mins.
Exp. 5:			
50 c.c. whole milk	1·0	3·7 c.c.	30 mins.
50 c.c. whole milk (no sediment)...	1·0	2·9 c.c.	30 mins.
Sediment from 50 c.c.	1·0	0·4 c.c.	30 mins.

From this table it will be seen what extremely variable results were obtained with this almost or completely sterile milk.

Much more oxygen was collected by the method finally employed (with the gas apparatus devised by Harden, Young and Thompson) than by any other method used by us. For every 50 cc. milk, 5 cc. of a 1 per cent. solution of H_2O_2 were used, this quantity of milk being much greater than that used by previous observers, so that if a comparison is made between the amounts, this must be allowed for as well as for the fact of the different apparatus.

Before leaving the question of the source of the catalase in cows' milk, the work of Bordas and Touplain must be mentioned; and this rather because of the attention which their work excited, than because of its value for the present report. They apparently looked upon peroxidase and catalase as the same body.

They obtained evidence which led them to believe that the reactions were not destroyed by boiling, and that they were due to the presence of calcium-caseinogenate.

Sarthou (1909) wrote against this view, and pointed out that they gave the reaction for peroxidase and not for catalase.

J. Meyer([110]) (1910) also wrote showing that the catalase reaction is positive if caseinogen or casein be prepared by means of trichloracetic acid, heat or acid, and then mixed with water. If, however, the milk is previously boiled, then no catalase reaction is given. The boiled milk can, however, be re-activated by platinsol.

The work of Grimmer([70]) (1910) may also be mentioned. This worker extracted the mammary glands of cows' and other animals with glycerine, and found that the catalase content was greater in non-lactating than in lactating glands, but that even in this case it was much greater than in the milk.

Reviewing the work described in this section, it appears that--

1. Catalase is constantly present in cows' milk.
2. That the main source of this catalase in milk is bacterial.
3. That it is found in small quantities in milk collected free from bacterial contamination.

Section 2.—The Estimation of Catalase.

It is quite clear that if the amount of catalase is to be taken as representing the degree of contamination, some method must be adopted of measuring accurately the amount of oxygen evolved by the sample of milk under examination. This, although apparently simple, has proved a difficulty which can hardly be said to have been successfully overcome.

The apparatus must be simple so that it can be easily used in a dairy laboratory; the test must be carried out as quickly as possible, and it must be reliable. A number of pieces of apparatus have been devised for this purpose, of which only the more important will be described.

Thus Koning([88]) used a saccharimeter tube, and read off the results obtained.

Burri and Staub([48]) introduced a method wherein a small vessel, having a long graduated neck, was used. The neck was filled with a plug of agar-agar, and the milk and hydrogen peroxide inserted through the opposite end. The gas, as it was evolved, pressed up the agar plug, and the amount of oxygen could then be read off.

The Lobeck-Gerber([66]) apparatus is the one which is advertised in most catalogues dealing with dairy apparatus. It is a neat, and rather pretty piece of apparatus, and on the surface it seems that it should be accurate.

In some of these methods the essential fact is entirely neglected, namely, that the apparatus must be capable of being frequently shaken during the whole period of investigation.

Kooper([94, 95]) (1911) endeavoured to reduce the confusion existing in this direction; he tried to find some constant factor by which the results obtained by one method might be multiplied, so as to make them numerically comparable with those

obtained by another method He used four different apparatus, namely, Koning's, Funk's, Henkel's and Gerber's (Lobeck's). Of these Koning's and Henkel's cannot be shaken, Funk's can be shaken, but the gas is mixed with the milk; Lobeck's also can be shaken, and gives the highest reading of the four; this does not, however, necessarily mean that it is the most accurate.

Kooper seems to have carried out this most laborious task with great care, and, working upon a large number of samples, of which he took the average, he believed that he had found factors by which it would be possible to convert the results obtained by one apparatus into terms of another. The figures obtained by him upon which he based his average figure, showed such high differences that it seems doubtful whether the results obtained by this method possess any value. His paper gave rise to a prolonged discussion between himself and Grimmer upon the details of his methods, and the results obtained, into which it is not necessary to enter.

Spindler([177]) (1911) used Lobeck's apparatus and found that the amount of gas evolved depended upon the capacity of the apparatus, also upon the temperature, rather more gas being formed at 37° C. than at 25° C. Further the amount of H_2O_2 used had an appreciable effect. As a whole 15 cc. milk and 5 cc. of 1 per cent. H_2O_2 gave the best results, as to absolute quantity; but smaller amounts of milk and H_2O_2 in the same proportions gave relatively larger amounts. Successive additions of 5 cc. of H_2O_2 gave still larger amounts of gas.

Harden and Lane-Claypon([75]) (1912) found that neither Koning's nor Lobeck's apparatus was reliable, but apparently satisfactory results were obtained by using the apparatus devised by Harden, Thompson, and Young for the investigation of the gases evolved by growing yeast. This apparatus requires constant shaking, the evolved gases passing into a eudiometer, and being collected over mercury.

Although there are these difficulties connected with the estimation of the oxygen produced by catalase, yet it may reasonably be supposed that when the same apparatus shows widely diverging amounts of oxygen from different samples of milk, different degrees of catalase content are present. So that, although much of the work cannot be taken as absolutely accurate, it would, I think, be unjustifiable to suppose that no reliance can be placed upon the results, provided the minor degrees of difference are neglected.

Section 3.—The relation of the catalase content to the condition of the milk.

The condition of milk may be affected by the state of the gland before secretion, and by contamination after milking.

The conditions of the gland which show departures from the average normal composition of the milk occur in the early days after parturition, and when the gland is " drying " or is inflamed, as in mastitis.

Bier (1905) showed that the catalase was present in colostrum, but he found the amount variable. Giffhorn([67]) (1910) found the content increased in mastitis.

Gerber and Ottiker (1910) found that the catalase content of the milk varied with the breed of the cow, the feeding, and general conditions of all sorts. That it was increased in colostrum, in the milk from a gland when nearly "dry" and in disease of the udder.

Kooper([94]) (1911) also found that the breed of cow affected the catalase content.

Spindler([177]) (1911) confirmed the work of Gerber and Ottiker, and showed that the catalase content is increased in all inflammatory conditions of the udder, and when the gland is in either the early or late stages of lactation, that is when the gland is not in full activity.

These facts agree closely with those obtained for human milk (*see* pp. 23, 24).

The relationship of the bacterial count has been investigated by many observers, but the concensus of opinion is, that although the catalase content is increased by bacterial contamination, there is no definite relationship between the actual number of bacteria and the catalase, although a rough estimation has been suggested by some observers; this last does not apply to small numbers of bacteria (*cp.* Jensen, p. 18).

Lam([97]) (1906) tried to work out a standard of the amount of oxygen which should be evolved by milk, with varying degrees of contamination. He used 10 cc. milk and 5 cc. H_2O_2 (1 per cent.) and considered that normally good milk should give off from 0·3-1·0 cc. O_2, and that if more is evolved the milk is not good. Excess of catalase being given either by stale milk, or by milk obtained from unhealthy cows.

Gerber and Ottiker([66]) (1910) gave a higher figure than Lam as being the average amount of oxygen which should be evolved. They used 9 cc. milk and 3 cc. 1 per cent. H_2O_2, and obtained from 2·5-3·0 cc. O_2. More than this amount was probably due to dirt in the milk or to disease of the cow.

Heygendorff and Meurer([78]) (1910) and Schroeter([161]) (1911) all failed to find any definite relation between the bacteria, and the amount of oxygen evolved.

The irregularity of the figures is well illustrated by some of Schroeter's figures:

Bacterial count.	Sediment figure.	Oxygen given off.
11,775,000 ...	·25 ...	6·5 cc.
12,000,000 ...	·3 ...	2·25 cc.

He thought there was some relation between the sediment and the amount of catalase. This might presumably be either due to leucocytes or to dirt.

The evidence as a whole shows that there is increase of catalase in disease of the gland, or in gross contamination. The estimations of catalase which are carried out in some daries for the detection of these points are apparently only moderately reliable, and of little or no value unless there is a considerable excess of catalase, in which case it may be useful.

On the Presence of Catalase in Human Milk.

Raudnitz[133] (1898) found catalase in human milk, as did also Luzzati and Biolchini[38] (1901).

Marfan and Gillet[109] (1902) obtained an evolution of gas from milk on the addition of hydrogen peroxide.

Friedjung and Hecht[64] (1903) worked upon a large number of samples of human milk. They found the amount of catalase extremely variable; in some samples 2 cc. of milk only gave a trace of oxygen whilst the same amount of other samples gave as much as 4 cc. As a whole the healthier the woman and the freer the milk-flow the less the amount of catalase. Milks of colostral type, rich in cells, give the highest amount of O_2; the time of disappearing of the colostral type of milk after birth was very irregular. The last portion of milk taken from the gland was richer in catalase than the rest. The amount of catalase is increased in some constitutional diseases, and in mastitis, but is very variable. Young mothers have perhaps rather less catalase than older ones, but the number of previous pregnancies seemed to be without influence.

Jolles[84] (1904) found that human milk contained more catalase than cows' milk, but that the amount was very variable, and that one breast may contain more than the other.

Bier[37] (1905) found great differences in the catalase content of the milk of the same woman. It was sometimes absent, and the amounts obtained when it was present was stated to have varied from 3—218 cc. O_2. Colostrum had a higher power of splitting H_2O_2 than normal milk. He states that the blood of a wet-nurse contained about 10 times as much catalase as the milk.

R. van der Velden[199] (1907) carried out a large number of experiments with human milk. The milk was collected by means of a breast-pump, and every precaution was taken to ensure sterility. 5 or 10 cc. milk and either ·5 or 1·0 cc. 30 per cent. H_2O_2 were taken for each experiment. The mixture was shaken for 10 minutes by means of a shaking machine worked by a turbine, the gas being collected in a gas-burette.

The amount of catalase as determined by the oxygen evolved, was found to vary from day to day, and was different in the two breasts at the same time. There was also a great difference between the amounts given by different women. Sometimes there was sufficient catalase to give off 13 cc. O_2 and sometimes only 1 cc., in 24 hours. As a whole, colostrum was richer in catalase than the milk of later lactation periods.

V. d. Velden also tried to ascertain whether there was a relationship between the amount of catalase and of the other constituents of the milk. There appeared to be some degree of connection between the cell-content and the catalase, but little or none between the fat content and catalase. In the fresh carefully-collected milk there was no connection between the bacterial count and the catalase, but if the milk was allowed to stand for 24 hours, then a certain degree of relationship became apparent.

Clinically it was found that the infants of mothers whose milk contained little catalase throve just as well as those whose mothers gave more—nor could any difference be detected between the health of the infants and the daily variation in the catalase content of the same milk.

A. Torday([186]) (1907) thought that catalase in the human milk examined by him, was probably connected with the fat since the cream was richer in catalase than milk alone, and this again than lactoserum.

E. and A. Torday([187]) worked together upon the catalase content of human milk obtained from women in a lying-in ward. The milk was collected with all precautions, and on plating out was nearly always found to be sterile. They found that there was no relationship between the age of the woman, the length of lactation, nor the number of the pregnancies. Pre-natal colostrum was very rich in catalase. As a whole the milk of a weakly woman was richer in catalase than the milk of a healthy one, and in that from a gland which was acting feebly, than in that from one in full working order. They considered the catalase content to be far too variable for it to be any indication of the nutritive value of the milk.

Summarising it appears—

1. That catalase is almost always present in human milk, but the amount varies within very wide limits.
2. That no connection has been traced between the amount of catalase in the milk and the age of the woman, the number of the pregnancy, or of the age of lactation, except in the case of colostrum, where it is increased.
3. That the healthier the woman, as a whole, the less the catalase.
4. The variation in the catalase content does not appear to affect the child in any way.

Catalase is also present in goats' milk, as has been shown by various observers, and it was found by Harden and Lane-Claypon to be present in rather larger quantities in milk collected by catheter, and sterile, than in cows' milk similarly collected.

3. Reductase.

The reductases are bodies whose action consists in bringing about the chemical reduction of a given substance.

Their action is most easily detected if the substance used changes colour as a result of the reduction. For this reason methylene blue has been found satisfactory, and has been largely, although not exclusively, used in the investigation of the presence of these ferments in milk. Methylene blue has been used alone—in which case, if it is reduced, the ferment or body causing such reduction is known as a "*direct reductase.*" It has also been used with formalin and the resulting reduction is said to be brought about by an "*indirect reductase.*" This last body has also been called "aldehyde-catalase," "aldehyde-reductase," "formaldehydase," and it has also been suggested by Bach ([19, 20]) that, following the general type of nomenclature,

"redukase" would be more appropriate. Further, inasmuch as the reaction was first discovered by Schardinger([157]), it is often known as "Schardinger's reaction," and on the assumption that the body is a ferment it is known as "Schardinger's ferment." These two reactions are most frequently denoted by the letters M.B. (methylene blue) and F.M.B. (formalin-methylene-blue) respectively.

In addition to these two substances some authors have dealt with a third reducing agent, *hydrogenase,* which converts sulphur into sulphuretted hydrogen. This action will be considered after the direct and indirect reductases.

Direct and Indirect Reductases in Cows' Milk.

Vaudin([198]) (1897) first showed that milk when fresh was capable of reducing indigo, and that the older the milk the more rapid the reduction; he considered that the rate of reduction depended upon the presence of bacteria.

Neisser and Wechsberg([119]) (1900) working upon the reducing power of tissues found that methylene blue was reduced by milk, and they considered that this phenomenon might be used for the determination of the bacterial content of the milk.

Wynter Blyth([207]) (1901) showed that fresh milk will reduce litmus.

In 1902 Schardinger([157]) published his first paper upon the reducing properties of milk, and it has been the starting point of an immense amount of work by other observers, and of much controversy. Schardinger showed that milk had the property of reducing methylene blue in the presence of formalin; the solution used for this reaction was made up of—

5 cc. saturated solution of methylene blue,
5 cc. formalin,
190 cc. water,

and this solution has been used by all subsequent observers who have worked upon this subject, and is known as Schardinger's reagent or solution.

The solution of methylene blue used by him, was made up of—

5 cc. saturated solution of methylene blue,
195 cc. water,

and this has been used by most subsequent observers, and is generally described as "methylene blue solution."

Schardinger found that when milk was quite fresh the solution which contained formalin was more quickly reduced, while later on, when the milk was older, the reverse was the case. The rate of the reaction apparently depended upon the age of the milk, and the number of bacteria present.

He concluded that one of two things must happen:—

1. Either an aldehyde-like substance was necessary for the reduction of the methylene blue; this was gradually formed in the milk by bacteria, but could be replaced in the early stages by formaldehyde or some other similar substance; or
2. The reaction was due to the "living protoplasm" of the bacteria.

This last he considered most likely to be the true explanation. In this he agreed with Cathcart and Hahn[71], who in the same year showed that many bacteria had reducing properties towards methylene blue, and that the property was probably attached to the protoplasm of the cell.

Schardinger also found that the optimum temperature for the reaction with formalin was 45-50° C., the colour usually disappearing within 30 minutes, after incubation. Temperatures of 20-23° C. were also used.

After the publication of this paper it became generally recognised that the reduction of methylene blue, and of formalin and methylene blue, were different reactions, and inasmuch as Schardinger's reagent was capable of being used in fresh milk the more quickly of the two it seemed that it would be valuable for the differentiation of raw and heated milk.

Utz[189, 190] (1903 and 1904) pointed out that the results obtained with Schardinger's reagent were dependent upon the reaction of the milk. In fresh, slightly alkaline milk it was positive, but in stale milk which is acid in reaction, it was negative, but could be restored even in sour milk by the addition of sufficient alkali. He believed that the lactose of the milk played a part in the reaction.

Siegfeld[171] (1903) also believed the reaction to be unreliable for the detection of raw or boiled milk.

Rullmann[147] (1904) showed that the reaction with Schardinger's reagent was positive even after heating at 68° C. for one hour, but it was negative if the temperature was raised to 71° C. Methylene blue alone was negative at both temperatures. Upon this Utz[193] again wrote emphasising his point about the reaction. Both Rullman and Utz were dealing with the subject solely as a means of differentiating between raw and boiled or heated milk, and there seems to have been some degree of confusion between oxidising and reducing actions, into which it is not necessary to enter.

Schardinger[158] wrote vindicating the reliability of his reaction as a test for boiled milk, and the discussion continued for some time longer upon these lines; the main questions being—

1. Was it a reliable test, or did it lend itself to falsification of milk by the addition of an alkali; and
2. Was it as reliable as the peroxidase reaction (*see* pp. 6, 7), and if so, was it as easy to carry out?

This aspect does not, however, directly concern this report.

The next phase in the investigation of the reductases was now entered upon. It was concerned with the *cause* of the two reactions with methylene blue. Were the two reactions due to the same cause? If so were they due to ferments or to bacterial action? If the former then they might be of biological significance, and if the latter then they might be used as a means of estimating the degree of contamination of the milk.

Smidt[172] (1904) showed that there were three factors in milk which could bring about the reduction of methylene blue: (he appears to have regarded this action as a catalytic one).

These three factors were:—

1. Lactose, or other substances which became alkaline on boiling,
2. Ferments, and
3. Bacteria.

Smidt showed (*a*) that fresh milk a few hours after milking gave a positive reaction with Schardinger's reagent but not with methylene blue alone. The reaction with formalin-methylene blue was weakened by heating to 70° C. and destroyed by heating to 75° C. for 20 minutes. He considered this reaction to be due to a ferment which he called aldehyde-catalase.

The rate of reduction of methylene blue alone, depended upon the number of bacteria present and was in direct relation to it.

He also showed that lactose in alkaline solution at 45-50° C. gives decolourisation in a few minutes and methylene blue is reduced when milk is boiled for about 15 minutes, provided the dye is added at boiling temperature or before boiling.

He therefore considered that the reaction with formalin-methylene-blue was different in origin from that with methylene blue alone.

In effect these views of Smidt's have been almost universally acknowledged to be correct, although a vast amount of labour has been bestowed upon the subject since Smidt's paper appeared.

(In accordance with the practice in most of the work after this period, the terms F.M.B. and M.B. will be used to denote Schardinger's reagent and methylene blue alone respectively.)

Seligmann([165, 166]) (1905 and 1906) published several papers dealing with the cause of these two reactions, and attributed both of them to bacteria. Throughout his work, however, I have been unable to find any indication of the age of the milk used, of the quality of the milk and of the precautions taken in collecting it, nor do any bacterial counts appear to have been made.

In some of the experiments antiseptics were used but a germ-free fluid does not appear to have been obtained in any case.

Seligmann found that on the first day after milking only the F.M.B. was reduced, and on the second day both were reduced, whilst finally F.M.B. was sometimes reduced more slowly than M.B. He also found that milk treated with formalin took longer to reduce F.M.B. than raw milk.

These facts he interpreted as showing that formalin reduced the number of bacteria present, and hence increased the length of reduction time. Also he boiled milk for an hour and found that it had completely lost its reducing power, but if inoculated with sour milk, it regained the property of reducing both F.M.B and M.B. and hence both were concluded to be bacterial in origin. The same result was obtained with milk heated to 100° C. and incubated for 24 hours; that is, reduction occurs with both reagents when the surviving spores or bacteria have multiplied.

Seligmann further believed that the reduction of methylene blue was brought about by the decomposition products of casein formed by the action of bacteria, the deficiency of these products in the early hours after milking being made good by formalin, which assisted the reduction of the methylene blue.

He isolated a bacillus which he found had strong reducing powers for F.M.B. and thought that the reduction of F.M.B. by

pure fresh milk might be due to the bacteria which had been present in the udder. Seligmann carried out a number of other experiments upon the same lines, but it cannot be said that they are all of them convincing, some of them being capable of an opposite interpretation from that put upon them by him, especially those dealing with the reduction of F.M.B. Evidently the methylene blue in the F.M.B. is capable of reduction by bacteria in the same manner as in the M.B. solution.

Seligmann showed that if milk was centrifuged the body responsible for the reduction of F.M.B. was carried up with the cream, at any rate for the most part, but could not be washed out of the cream. The reduction times obtained were:—

22 minutes for skim milk.
8 ,, ,, whole milk.
5 ,, ,, cream,

and watery extract of cream did not reduce at all.

He obtained evidence of reduction with precipitated caseinogen, and the reducing substance could not be separated by watery extraction.

Jensen[83] (1906) definitely separated the two reactions with F.M.B and M.B. under the terms direct and indirect reductase.

He showed that a large number of the organisms of milk will produce a direct reductase, the acid-forming organisms bringing about reduction rather more slowly; further that there was no relationship between the reducing and catalytic powers of these organisms, and that therefore these reactions are distinct and due to different properties.

Really pure milk did not reduce M.B. but only F.M.B., and he points out that Seligmann's own work showed the same, as has already been mentioned.

Jensen found that the intensity of the action of F.M.B. depended upon the fat content, the strippings or last portions of the milk reducing much more rapidly than the first milk. He agreed with Seligmann that the reducing body cannot be washed away from the cream and considers that it does not therefore come from the leucocytes.

The following table shows the results obtained by Jensen very clearly, and it also shows that there is no connection between the bacterial content and the reducing power to F.M.B.

Milk.	Bacteria per c.c.	Fat Content. Per cent.	Reduction Time of F.M.B.
1st Experiment:			
First milk	16,000	·55	Not in three hours.
Middle milk...	480	2·70	120 min.
Strippings	360	8·30	15 ,,
2nd Experiment:			
First milk	3,200	1·5	90 ,,
Middle milk	2,800	3·40	75 ,,
Strippings	360	7·80	14 ,,
3rd Experiment:			
First milk	—	1·70	105 ,,
Middle milk...	—	3·35	80 ,,
Strippings	—	6·40	16 ,,

Butterberg([19]) (1906) showed that Schardinger's reaction was negative if milk was heated to 70° C. for 20 minutes, and that in all cases where the M.B. reaction was positive, the bacterial count was high.

Brand([44]) (1907) found that F.M.B. is reduced best at a temperature of 70° C., but that the reaction becomes negative if the heating is prolonged. M.B. on the other hand is not reduced in fresh milk at 70° C., but is produced in boiled milk if the milk is inoculated with fresh milk and subsequently incubated. It is then reduced at 50° C. but not at 70° C. Brand concludes from these experiments that M.B. and F.M.B. are distinct reactions, of which M.B. is due to bacteria and F.M.B. to a ferment.

As regards the distribution of the ferment, Brand found that although the major part of the ferment goes up with the cream some is left behind in the milk, and will give a positive reaction at 70° C. He also showed that the addition of alkali aids the F.M.B. reaction, and that the reaction can be reproduced in boiled milk by this means. Commercial falsification could, however, be detected by the fact that the boiled control would also be positive.

Monvoisin([112]) (1907) also showed that although most of the ferment was precipitated by saturation with magnesium sulphate, yet some remained behind in the filtrate and hence it was not fixed to the fat globules.

Koning([88]) (1907) in his admirable studies on milk showed that there are a large number of organisms which, if inoculated into sterile milk and incubated for 24 hours, gave reduction of methylene blue; among these were B. Subtilis, B. Coli Communis, B. Fluorescens Liquefaciens and Non-Liquefaciens, B. Prodigiosus, B. Mesentericus, and some lactic acid producing organism. The time of reduction varied greatly with the different organisms, the conditions of experiment being kept constant.

He also showed that F.M.B. is reduced by sterilised and boiled milk on the addition of lactose or alkali, and that the reduction is more rapid at 100° C. than at 45° C., but is not so rapid as the ordinary reduction with raw milk.

Koning found that colostrum reduced F.M.B. more slowly than ordinary milk, but that pathological milk gave a more rapid reduction. He points out the difference in reduction time between the first milk and the strippings, and gave the following table:—

—	Cow I.	Cow II.	Cow III.	Cow IV.
First milk reduced F.M.B. in ...	30	12	11	42 minutes.
Middle „ „ ...	6	6	6	42 „
Strippings „ „ ...	2	4	4	10 „

He found also that heating for one hour at 65° C. was sufficient to destroy the F.M.B. reaction in raw milk. He concludes that the reduction of F.M.B. is due to a specific ferment.

Smidt([173]) (1908) again wrote upon the question of the differentiation of the M.B. and F.M.B. reactions. He found, as Seligmann, Brand and Jensen had already done, that the reductase for F.M.B. goes up in the cream on centrifuging, and that it cannot be washed away from the cream. This he thinks is against its bacterial origin, and indeed the table given by Jensen (*see* p. 28) shows very clearly the absence of connection between the reducing power to F.M.B. and bacterial content.

Skim milk incubated for 24 hours gave a shorter reduction time for M.B. than for F.M.B. Also pure, almost bacteria-free, milk if kept on ice at first only gives the F.M.B. reaction, the time of which gradually lengthens as the milk is kept. Subsequently in the course of a few days the M.B. reaction becomes positive, doubtless due to the effect of the formalin in preventing bacterial growth gradually wearing off.

Smidt did not accept Seligmann's idea of the part played by the degradation products of caseinogen in bringing about reduction. Milk as it leaves the udder will only reduce F.M.B., and this is accomplished in about 10 minutes at 45° C. The reducing substance must therefore be present in the udder, and as milk as it leaves the udder is sterile the F.M.B. reaction cannot be due to bacteria. Further, samples of milk treated with chloroform or phenol, and which on plating out were found to be sterile, gave the F.M.B. reaction even after standing for 24 hours.

Seligmann([167]) (1908) replied, defending his position, and maintaining that M.B. and F.M.B. were both due to bacteria. He argues that milk which has been kept reduces both reagents in the same time, and if milk be incubated the reduction time for Schardinger's reagent may even be less than for the methylene blue alone; further, that the addition of formalin to milk increases the reduction time in both cases, and that as the effect of the formalin wears off the reduction time for both decreases, thus pointing to the primary increase in the reduction time being brought about by the inhibitory effect of the formalin upon the bacterial growth.

Seligmann's arguments in favour of his views do not seem to be very conclusive, because he does not consider the period at which there is the most marked distinction between the two reactions, namely, in the first few hours after milking. It must also be borne in mind that in the presence of bacteria bringing about the reduction of methylene blue these same organisms would also presumably reduce the methylene blue present in Schardinger's reagent, since the formalin could hardly be considered as inhibiting the bacterial effect during the period of the reduction time.

S. Oppenheimer([128]) (1908) obtained the typical F.M.B. reaction in both fresh and stale milk at 70° C. In fresh milk,

collected with all possible precautions against bacterial contamination, the reduction of F.M.B. took place in from 10-15 minutes at 50° C. and in 7¼ minutes at 70° C. He found no connection between the rate of reduction of F.M.B. and the bacterial content, but he showed that there was a connection between the fat content and the rate of reduction, since the first part of the milk which was poor in fat had a longer reduction time than the rest of the milk. He considers that the reduction of Schardinger's reagent is due to a ferment.

It seems clear that the reduction of methylene blue alone can fairly be attributed to the unaided action of bacteria, and it is not therefore necessary to continue to bring forward evidence in support of this point; at the same time, since this report is intended to deal with the most important papers upon the whole subject, it seemed advisable to mention briefly the work both of Barthel, P. Sommerfeld and Schroeter which deal chiefly with the reduction of methylene blue and of neutral red respectively by the agency of bacteria in milk.

P. Sommerfeld([176]) used neutral red in his experiments upon the reducing power of milk. This reagent gives a strong red reaction with lactic acid and becomes yellow on reduction.

Sommerfeld found (1) that market milk gave reduction in all cases in from 7-20 hours; (2) that in milk collected with all precautions so that the bacterial content was only from 1,000-2,000 per cc. reduction did not occur until the bacterial content had increased considerably; and (3) if milk be heated the reducing power is destroyed, but this returns upon inoculation. With about 200 bacteria per cc. the reduction took 44 hours at 37° C., and with 1,000,000 per cc. about 12 hours at the same temperature. He concluded that the reduction is due entirely to bacteria.

Barthel([24, 25, 26]) published three papers (1908, 1910 and 1911) which it will be convenient to take all together at this stage. Essentially he was endeavouring to obtain a measure of the contamination of milk by means of the reduction of methylene blue. He first of all took milk and sterilised it by means of toluol or chloroform, and confirmed the sterility by plating out. This procedure had little or no effect upon the F.M.B. reaction but prevented the reaction with M.B. If, however, acid was added to fresh milk the reduction of F.M.B. was delayed but not that of M.B. Barthel considered that Schardinger's reagent could not be used as a means of testing for contamination.

Summing up the results obtained with M.B., Barthel considered that if milk was of good quality it should not reduce M.B. in less than three hours. If the reduction time was from 1-3 hours the milk was only moderately good, and if the reduction was effected in less than one hour the milk was bad. He found, however, that there was no direct relation between the absolute number of bacteria and the rate of reduction, and that the method with M.B. was more sensitive at a temperature of 38-40° C. than at 45-50° C.

The absence of direct relation between time of reduction and bacterial content is well shown in the table given by Barthel,

which incorporates both his own results and some obtained by Jensen in work which has been already considered.

Acidity.	Decolorisation Time.		Bacteria per c.c.
	Barthel.	Jensen.	
15	9 hours	1 hour	7,200,000
15	5 ,,	1 ,,	9,215,000
17	7 ,,	1 ,,	5,760,000
15	6 ,,	1 ,,	3,840,000
15	6 ,,	1 ,, 10 min.	10,680,000
16	Approx. 7 ,,	1 ,, 10 ,,	4,450,000
16	,, 6 ,,	1 ,, 20 ,,	2,590,000
15	,, 9 ,,	1 ,, 45 ,,	1,860,000
17	,, 9 ,,	2 ,, 30 ,,	3,710,000
15	,, 7 ,,	2 ,, 30 ,,	7,190,000

(This table is taken from the paper published in 1911.) The acidity was estimated by Thörner's method.

Schroeter([161]) (1911) investigated the relationship between the number of bacteria and the rate of reduction of methylene blue, and was, like Barthel, unable to obtain any direct result. He used 40 cc. of milk and 1 cc. of methylene blue solution at a temperature of 38-40° C. He thinks that if the reduction time exceeds seven hours there will probably have been less than 1-1½ million bacteria per cc., and if reduction is effected in less than two hours there will probably have been more than 1-1½ million bacteria per cc. It appears, therefore, that the degree of contamination by bacteria is not easily measured by this means.

It may perhaps simplify the somewhat complicated details of these two reactions if the position of the matter at the end of 1908 be reviewed and briefly summarised.

It had been shown beyond much possibility of error that milk contains two substances capable of reducing methylene blue. Of these one reduced methylene blue directly, the action being due to the bacteria present in the milk. The other reduced methylene blue only in the presence of formalin, and the action was probably due to a ferment or other substance present in the milk; that this substance was connected with the fat of the milk, but the amount of this substance had no direct relation to the amount of fat present.

After this period the attention of observers was directed to the latter of these two reductions that brought about by Schardinger's reagent. From 1908 up to the present year and even in this year, papers have appeared in very considerable numbers, and a truly large amount of time and labour has been devoted by many observers to this question. It is now necessary to deal with this work in further detail.

Schern([159]) (1909) worked upon the presence of the ferment in different stages of lactation. He found that the reaction is frequently negative during the early weeks of lactation, becoming positive later on. This is in accordance with Koning's work on colostrum already referred to (*see* p. 29). The reaction is always positive in cows which have been milked for some months, the

F.M.B. being decolourised in about 10 minutes at 65-70° C. When the reaction is just beginning to appear in the early stages of lactation then a better reaction may sometimes be obtained at 45° C. He also showed that serum has a well marked inhibitory effect upon the reaction. The serum of many animals was examined and the same result obtained with them all.

In this connection it may be mentioned that Giffhorn([67]) in 1911 showed that if F.M.B. was decolourised with very great rapidity in fresh milk the milk will have come from a diseased udder, which also agrees with Koning's observations.

Trommsdorff([188]) (1909) obtained milk direct from the cow's udder by means of a milking tube. By taking the most stringent precautions against contamination he was able to obtain milk which, on plating out, was found to be sterile. This milk was tested for the presence of Schardinger's reaction and the result was positive. Hence it seems certain that milk which has never been contaminated by bacteria does contain a substance capable of reducing methylene blue in the presence of formaldehyde. Trommsdorff found that the acidity of the milk rose during the course of the reaction, and concluded that this was due to the formation of formic acid from the formalin. He found, however, that the acidity rose in precisely the same manner, whether formalin was added or not, so that it was not due to the production of formic acid.

Kooper([91]) (1910) endeavoured to show that the F.M.B. reaction was bacterial in origin. He took milk soon after milking and on the two following days, and using Schardinger's reagent only, found that the reduction time was shortened as the days passed, and that boiled milk regained its reducing power if small quantities of raw milk were added to it. The same remarks apply here which have already been made in regard to Seligmann's work, and it need not be further dwelt upon (*see* p. 30).

Sames([150]) (1910) working alone and also in conjunction with Römer([141]) brought out many points in connection with this reaction. They investigated the reducing power of the different portions of milk as it leaves the udder. They found, as had in part been done by previous observers, that the first milk very rarely reduced at all, and then only incompletely. Middle milk varied a great deal in its reducing power, and did not always reduce completely; whilst the strippings always reduced very rapidly, never taking longer that $8\frac{1}{2}$ minutes. The various constituents of the three different portions of milk were then most carefully investigated, and it was found that the only constituent which varied markedly in quantity in the different portions was the fat, the content of which was very appreciably higher in the strippings than in the rest of the milk. There was, however, no constant parallelism between the rate of reduction and the percentage of fat. If the cows were kept waiting long between the periods of milking then the reaction was often negative.

As to the relation of the reaction to the age of lactation they were unable to obtain any connection; it was sometimes absent, and when present varied in strengthening the same cow from day to day.

They consider that Schardinger's reaction is essentially due to the activity of the gland, and that the reacting material is derived from the break down of the gland tissues; it can only be considered as due to a ferment if the contents of the gland cells and their excretion products can be called such. Römer and Sames further point out that Heidenhain said that end milk was formed by the break down of cells and Koning (*see* p. 29) showed that pathological milk, which presumably contains cells or their debri, reduced F.M.B. very rapidly. They also point out that this body will not bear dilution, which a ferment will. This substance is destroyed by dialysing for two days, whereas the oxidising ferments of milk are not destroyed by four days dialysis.

The authors also deal with the reduction obtained by Koning at 100° C. This they believed to be brought about by the lactose in the presence of an alkali, a reaction which occurs equally well with boiled milk. If the caseinogen and lactalbumin be removed by the addition of acetic acid or by boiling, the filtrate containing the lactose will promptly reduce F.M.B. on the addition of two drops of $\frac{N}{1}$ NaOH.

Römer and Sames further showed that milk which has been heated or which has never given Schardinger's reaction will do so at once if 0·3 cc. of a one per cent. solution of ferrous sulphate be added; if, however, the ferrous sulphate is boiled either before or after its addition to the milk the reaction is negative. The addition of ferrous sulphate to raw milk accelerates the reaction, but is not the only cause of it, since ferrous sulphate in watery solution does not reduce F.M.B.

Sassenhagen([151]) (1910) did not obtain the F.M.B. reaction with colostrum, although he found that traces could be detected in the cream of the later colostrum.

Reinhard and Seibold([137]) (1911) collected milk both with a milking tube and also with all aseptic precautions without a milking tube. They investigated the effect of the age of lactation upon the presence of the ferment, as had been done by Schern, and Römer and Sames.

They found that in the early weeks of lactation the reaction was often negative, and that the time of its appearance was very irregular, thus confirming Schern. They also, however, found that in some cases where the reaction appeared negative it could become positive if the cream alone were taken the optimum temperature being 45° C. In cows of from 2-6 months lactation the reaction was always positive, F.M.B. being decolourised in 5-12 minutes at 65° C. and being negative at 70° C. There is no anti-ferment present in the colostrum, for milk and colostrum gives a positive reaction more quickly than milk and water, as was shown by Koning for goats' milk (*see* p. 41). This may be due to a slight adjuvant action, but as Römer and Sames found

that the body causing the F.M.B. reaction will not bear dilution, it may also be that the dilution of the control of cows' milk and water is showing slight inhibition.

As regards the distribution of the reducing substance they found that the main part goes up with the cream, and that there is no parallelism between the fat content and the amount of the ferment. There may be much fat and little reducing power, and *vice versâ*. The strippings have the highest reducing power, and the authors agree with Römer and Sames that the reduction of Schardinger's reagent is due to the activity of the gland, and is brought about by a pre-formed enzyme. They showed that the reaction is not influenced by the sucking of the calf, and that different teats yield milk of different reducing power. The reaction is the same whether the milk be collected by milking tube or by milking in the ordinary way, and is not influenced by small numbers of bacteria; an absolutely sterile sample of milk which had never been contaminated by bacteria gave a quicker reduction time than milk containing 46 bacteria per cc. and similar numbers.

Working with milk from diseased udders the authors found that there was much variation in the quantity of the ferment, which varied with the degree of inflammation; if the inflammation was severe the reaction was generally either delayed or absent.

In connection with the question of the activity of the gland, the work of Grimmer([70]) (1910) may be mentioned. This observer took glands of cows and of other animals and extracted them with glycerine both before and after grinding with quartz. The glycerine extracts contained no substance capable of reducing either M.B. or F.M.B. Too much stress, however, should not be laid upon this fact since it is possible that the substance is not extracted by glycerine, or it may be that the ferment is only produced during active metabolism.

Rullmann ([148, 149]) (1911) published two papers upon the mechanism of the Schardinger reaction, and upon the question of its presence in sterile milk.

He showed that cultures of certain bacteria in sterilised milk will give reduction of both M.B. and F.M.B. and that formic acid could replace formaldehyde in the production of the F.M.B. reaction.

In a large number of experiments he showed that if lactose (20 cc. of a 5 per cent. solution) and NaOH (2 mg.) be added to either boiled or sterilised milk that great decrease can be obtained in the reduction time both of M.B. and of F.M.B. The formic acid reagent is also reduced but after a longer time. Lactose alone in watery solution will not reduce F.M.B., but the addition of ammonia, calcium or sodium phosphate to a mixture of lactose and soda brings about a great acceleration in the rate of the reduction, as does also a rise of temperature up to 95° C.

Rullmann also collected milk with a milking tube and obtained many samples of milk which were completely sterile, both broth cultures and agar plates showing no growth. In the sterile

samples the tests with M.B. alone were entirely negative whereas the reaction with F.M.B. was positive after a few minutes. (In testing with methylene blue alone the method of Smidt-Müller was used.) In the non-sterile samples methylene blue alone was gradually reduced with increase of the bacterial content. The temperature used for these reactions was 45-50° C. Rullmann found, however, that if milk is heated for prolonged periods to 68-70° C. the reaction is positive or delayed, being negative if the temperature is 70° C. or over. If the milk be heated several times to 100° C. and is kept long enough at that temperature the reaction will be positive.

He concludes that there is a substance present in milk which is capable of bringing about the reduction of Schardinger's reagent at 45-50° C. apart from bacteria, and that this substance is probably a ferment.

That reduction of the same reagent can also be brought about at higher temperature by the action of substances present in milk, *e.g.*, lactose and salts. That these substances probably assist in the reduction of the reagent at the lower temperature.

He also found that the milk from a diseased udder contained more reducing substance for Schardinger's reagent, presumably owing to increased cell-metabolism, bacteria, and blood filtration.

Before leaving the work of the year 1911 it may be of interest to deal very briefly with some work which has been carried out by various observers upon the purely physical aspect of Schardinger's reaction. This in 1910 Bredig and Sommer([45]) showed that platinum, iridium, and gold sols, will act in precisely the same manner as the milk ferment, and will reduce F.M.B. in approximately the same time, also that at 70° C. this reaction is greatly accelerated by the addition of alkali.

Formic acid can be used to replace formaldehyde in this reaction, the latter being oxidised almost exclusively to carbon dioxide by platinum sol.

The rate of the reaction depends upon the relative quantities of the aldehyde and of the ferment or sol. If aldehyde is present in excess the rate of reduction is decreased out of all proportion to the amount of the sol present.

Bach([19, 20]) (1911) dealing with the mechanism of action of the sols. points out that platinum sol. + hypophosphorous acid (H_3PO_2) + water (H_2O) gave H_3PO_3 (phosphorous acid) + 2H and this, if methylene blue be added, will give reduction, the temperature used being 70° C. The sol. can be replaced by the ferment and the hypophosphite by the aldehyde, according to the work of Bredig and Sommer just quoted.

Bach also showed that milk will reduce nitrates in the presence of acetaldehyde with the formation of nitrates; this reaction will not occur without the addition of the acetaldehyde, unless there is bacterial contamination, in which case reduction of the nitrate to nitrate is readily brought about without further addition.

Acetaldehyde and nitrate together give small quantities of nitrite but the rapidity and degree of the nitrite formation is very greatly increased by the ferment in the milk, and depends upon

the amount of the ferment which is present. The optimum temperature is 60° C.

Formaldehyde can replace acetaldehyde but is a much weaker agent.

Bach studied the same reactions with an extract of calves' liver, and found that the liver contained a substance giving similar reactions to those of the ferment found in milk, and presumably identical with it.

As regards the theory of the action Bach suggest the following interesting points: If water contains unsaturated ions of the form $H_2O <$ this may unite with either OH- or H- ions forming labile compounds, viz.;

$$H_2O < \begin{matrix} OH \\ OH \end{matrix} \text{ or } H_2O < \begin{matrix} H \\ H \end{matrix}$$

Now H_4O is the hypothetical sub-oxide of hydrogen and corresponds to M_4O where M is a metal. The other compound H_4O_3 is hydroperoxydhydrate.

Bach thinks that with platinum a compound $H_2O = O_2Pt.$ may be formed by the replacement of the two hydrogen atoms by one of platinum. By this means some of the $H_2O <$ ions would be removed and fresh ones formed so as to restore the original concentration of those ions.

Paal and Gerum([129]) had shown that this was the case for Palladium sol. and it seemed therefore probable that this would apply for Platinum.

Bach suggests that the ferment bringing about reduction should be called Redukase, rather than Reductase there being no reason for the insertion of the "t." Further that the particular ferment bringing about the reduction in the presence of aldehyde, should be called "perhydridase."

He considers that there is an analogy between the peroxidase and reductase reactions, viz.:—

Peroxidase + H_2O = O (gives the peroxidase reaction), and

Reductase + H_2O = 2 H (gives the Schardinger reaction, as in calves' liver).

It seems therefore that the action of the ferment in milk is almost if not entirely identical with that of the sols of the metals.

Before the appearance of the various papers upon this subject in 1911, in conjunction with Dr. Harden I had commenced some experiments upon the presence of ferments in sterile milk. Using milk obtained with a milking tube we obtained the same results as those of the writers previously quoted, namely, an absence of reduction with methylene blue, and the evidence of the presence of a substance reducing F.M.B., although the presence of this substance was somewhat irregular and uncertain.

Since the writing of this report was commenced, Burri and Kursteiner([47]) have reconsidered the whole question of the reductases in milk, and have reached conclusions similar to those of previous observers. These investigators did not use sterile milk, and were in consequence obliged to use disinfectants to get rid of the bacteria. They used chiefly phenol, and sodium

fluoride. They concluded that there is in milk no substance which reduces methylene blue alone apart from bacteria.

They investigated at some length the question of the formation of reducing substances in milk by boiling, and conclude that such are formed, if the boiling be sufficiently prolonged.

They found that the reduction time of raw milk and of milk which has been just boiled is almost identical, but that the rapidity of the reduction increases with the prolongation of the boiling. The experiments were carried out under anaerobic conditions since the authors found that the length of time of the reduction depended upon the amount of oxygen which was present. For instance if, after boiling or sterilising, the tubes containing the milk for experiment were placed upright and also sloping, the tubes which had been in the sloping position took longer to reduce than the ones which had been in the upright position, presumably because they had had more surface exposed to the air, and had taken up more oxygen. That this was the case the authors proved by the following means.

They took two tubes one small and the other larger, and joined these two together by an opening at one side, to which tubing was fixed. The tubes were closed with india rubber corks through which a glass-tube passed out to pyrogallic acid and potash. The small tubes were for the methylene blue and the larger tubes for the milk. The milk and the methylene blue were put into tubes when all was ready, and in one apparatus the two fluids were mixed; everything used being sterilised. Both apparatus were incubated at 90° C. after having been placed in connection with the pyrogallic acid and potash. The methylene blue in the mixed tube was decolorised in about four minutes, and the methylene blue and the milk in the other apparatus was then mixed, and became white in one minute. The difference was apparently due to the delay in getting rid of the oxygen in the apparatus, in which the mixing of the methylene blue and of the milk had already taken place before incubation.

Using this method the authors carried out many experiments upon the influence of the length of time of heating upon rate of reduction.

In one experiment where no disinfectant was used—

Raw milk gave reduction in	64 minutes.
Milk heated to 60° C. for 10 minutes reduced in	64 ,,
,, ,, ,, 90° C. ,, 10 ,,	,, ,, 60 ,,
,, just boiled	,, ,, 60 ,,
Sterilised milk	,, ,, 23 ,,

Incubation Temperature 38° C.

Further—

Raw milk	reduced in 68 minutes.
Milk just boiled	,, ,, 64 ,,
Milk boiled for 5 minutes	,, ,, 56 ,,
,, ,, ,, 10 ,,	,, ,, 52 ,,
,, ,, ,, 15 ,,	,, ,, 44 ,,
,, ,, ,, 20 ,,	,, ,, 35 ,,
,, ,, ,, 25 ,,	,, ,, 29 ,,
,, ,, ,, 30 ,,	,, ,, 13 ,,
,, ,, ,, 60 ,,	,, ,, 7 ,,

If different times are allowed to elapse between the first boiling and the testing, it is found that the difference between the reduction times is less marked; the shortening of the reduction time of the raw milk being found to be due to bacterial development and the lengthening of the reduction time of the boiled milk to the taking up of oxygen.

The authors discuss the question of the reduction of the F.M.B. solution, and conclude that it is due to a ferment (for which they suggest the name "formaldehydase"), whose action can be simulated by the addition of alkali or ferrous sulphate. It appears therefore that no appreciable amount of reducing substance is formed in milk by boiling milk, provided the boiling be not prolonged.

Römer([142]) (April 1912) wrote again summing up the points which he had shown in 1910 and dealing more especially with the value of Schardinger's reagent as a test for boiled milk. He points out that the reaction can always be made positive (*a*) by the addition of an alkali, which in fact is often done in any case, and (*b*) by the addition of ferrous sulphate in small quantities, as already pointed out by him.

He again remarks that the first milk is negative in reaction, and end milk positive: that raw market milk will often give a negative result and that the reaction cannot be said to be reliable as a test for the previous heating of milk.

Summary of the Causes of the Reduction of Methylene Blue by Cows' Milk.

1. The reduction of methylene blue by milk is due to bacterial contamination.
2. Reduction of methylene blue in the presence of formalin can occur in milk which has been obtained free from bacterial contamination.
3. The reduction of formalin-methylene blue is due to some substance, probably a ferment. This is almost, if not entirely, absent in colostrum, but can usually be found in milk after the early weeks of lactation; it is, however, very irregular in the time of its appearance, and in the quantity present.
4. First milk does not usually contain any of this substance, which is present in the greatest amount in the strippings or end milk; although the fat is the only constituent of the milk which varies greatly in percentage in the various portions (being increased in end milk), and although the ferment can for the most part be removed with the cream, there is no parallelism between the amount of ferment present and the fat content.
5. The action of this ferment can be replaced by alkali or ferrous sulphate, in small quantities.
6. The optimum temperature of reaction is from 45-50° C. the reaction becoming negative at a temperature of about 70° C.

7. The action of this ferment appears to be almost identical with that of the solutions of certain metals.
8. This reaction, apparently due to the presence of the same ferment, can be obtained with an extract of calves' liver, and
9. Reduction of either methylene blue alone or of formalin-methylene blue can be obtained in boiled or sterilised milk, provided the milk be boiled long enough. This reduction seems to be due to the formation of reducing substances, or to the presence of lactose and salts.

On the Presence of Reductases in Human Milk.

The amount of work which has been done upon the reducing substances in human milk is small, but enough has been done to obtain reliable information in this direction. It is much easier to obtain human milk free from any great degree of bacterial contamination than cows' milk, although the amount obtained is small.

Gillet([68]) (1902) using Abelous and Girard's method of testing for reduction by the conversion of nitrates to nitrites, obtained negative results. It has been shown that Bach obtained similar result with cows' milk (*see* p. 36).

Hecht([76]) (1904) who worked exclusively with human milk, obtained reduction of methylene blue alone, in from 1-2 days, but he was not sure that his samples were sterile, and does not appear to have controlled them by plating out. The time of reduction is entirely in accord with the reduction by bacteria, and by no other agency. In one case where special precautions were taken to ensure sterility, no reduction was obtained. Reduction was stopped by a temperature of from 60-80° C. Above this temperature, reducing powers again appeared on prolonged heating; presumably due to the lactose.

Hecht does not appear to have used Schardinger's reagent, and his results were most probably due to bacterial growth.

Rullmann([147]) (1904) obtained negative results with Schardinger's reagent, except in one case where he obtained a doubtful reaction.

Smidt([172]) (1904) who only used one sample of human milk, obtained a negative result.

Koning([88]) (1907) obtained no reduction of Schardinger's reagent with human milk.

P. Sommerfeld([176]) (1908), using neutral red, obtained no reduction with human milk, except in one case where there was inflammation, and cocci and leucocytes were present in large numbers. Nor did he obtain any reduction using methylene blue alone or in conjunction with formalin.

It appears therefore that *fresh human milk contains no reducing substances.*

On the Reductases in Goats' Milk.

Smidt([172]) (1904) was unable to obtain any reduction of Schardinger's reagent with goats' milk.

Koning([88]) (1907) was also unable to obtain any reduction of F.M.B. with goats' milk, although he thought it might contain a trace of a reducing substance, since mixed goats' milk and cows' milk gave a quicker reaction than cows' milk and water. The same remarks apply here, however, as in Reinhard and Seibold's work (*see* p. 34).

Harden and Lane-Claypon([75]) (1912) using milk obtained by milking-tube failed to obtain any reduction either of methylene blue or of formalin-methylene blue, even after many hours.

It appears therefore that *fresh goats' milk contains no reducing substances.*

Hydrogenase in Cows' Milk.

There remains one more substance to consider under the heading of reducing bodies; namely hydrogenase, or that ferment which has the property of forming sulphuretted hydrogen from sulphur.

The literature is not inconsiderable, and the results are quite conclusive. In 1891 Rösing([143]), in the course of his researches into the production of H_2S from egg-white, found that if sulphur was added to milk it was sometimes possible to obtain the reaction for sulphuretted hydrogen; the result was, however, sometimes negative.

Raudnitz([135]) (1902) in his excellent resumé of the literature deals briefly with the question of hydrogenase, and states that he himself had not been able to detect H_2S in milk, on the addition of sulphur; the same was also stated by Schardinger (1902).

Utz([192]) (1903) obtained H_2S from milk on the addition of sulphur after prolonged boiling.

Hoeffter([80]) (1904) like Rösing found that fresh milk sometimes gave reduction of sulphur; the negative samples, however, also reduced after they had been incubated for one or two days. The addition of antiseptics prevented this development, and as a result of these and of other experiments he concludes that the development of H_2S in milk upon the addition of sulphur is bacterial in origin.

Jensen([83]) (1906) also came to the same conclusion.

Brüning([46]) (1906) did not in any case find hydrogenase in fresh milk, but he found that if fresh milk was inoculated with stale milk that H_2S appeared on the addition of sulphur. He found that B. coli was especially active in the production of H_2S from sulphur.

Rullman([149]) using milk which was obtained sterile by means of the milking-tube was unable to detect hydrogenase in any case. He concluded that the formation of sulphuretted hydrogen from sulphur was bacterial in origin.

There can therefore be little doubt that the hydrogenase which has been found in raw milk is bacterial in origin.

Summary of the Work on the Reductases and on Hydrogenase in Milk.

1. Direct reductase, and hydrogenase, which have been found in fresh milk, are not present in milk as such, but are due entirely to bacterial contamination.
2. Cows' milk contains a substance, probably a ferment, which reduces methylene blue in the presence of formalin. The presence and amount of this ferment is, however, variable.
3. This substance is absent from human and goats' milk.

4.—On the Presence of Proteolytic Ferments in Cows' Milk.

The presence of ferments which break down proteins into their simpler constitutents has been investigated by several observers. The first authors to deal with the subject were Babcock and Russell, and Vivian.

Babcock and Russell([8, 9]) first prepared from milk a solution which had ferment activities. The solution showed the reaction for peroxidase, for catalase, and also had the power of hydrolysing proteins (*c.p.* Neumann-Wender, p. 16).

These activities were believed by the discoverers of this solution to be different manifestations of the same ferment, but they studied the proteolytic action exclusively. They were chiefly concerned with the ripening of cheese, which they believed to be carried out at any rate in great part by the ferment in question. They found that cheese could ripen whilst under the influence of ether, chloroform, &c., and that during the period of observation of such ripening (13-30 days), albumoses and peptones were formed from casein.

In their experiments the milk was carefully collected and was at once treated with an antiseptic, but no initial bacterial count appears to have been made. The authors point out that during the process of ripening the bacterial count decreases and yet the solution of casein continues. The ferment was more active in a neutral or alkaline medium, and was more powerful in the cream than in the rest of the milk, but the degree of activity was very variable, not only in different but in the same species of animal.

The products of activity of the ferment were ammonia, amides, and peptones, resembling (as the authors point out) rather the action of bacteria than of a ferment.

Freudenreich([63]) (1900) used milk rendered sterile with ether. In periods up to one month there was frequently no increase in the soluble nitrogen, but if the experiment was continued for longer periods up to 8 months an increase was obtained; the action did not appear to be carried as far as amino acids, and Freudenreich was, therefore, disinclined to believe that it had any part to play in the ripening of cheese.

Boekout and De Vries([39, 40]) (1899 and 1901) found that cheese made from pasteurised milk will not ripen, even after the addition

of the "milk-ferment." They took milk collected in the ordinary way, and milk collected under all possible precautions against contamination, and subsequently treated aseptically. The soluble nitrogen in this milk increases very slightly during the first few weeks of incubation, but not afterwards. The cheese made from this aseptic milk will not ripen, even though made by an experienced cheesemaker. If the soluble nitrogen be estimated in this form of cheese, and in ordinary cheese it was found that the gain in the former in about 4 weeks was from ·8 to 1·8 cc., and in the latter from 1·9-9·4 cc. of deci-normal alkali. The authors point out that even the aseptic cheese was not entirely free from bacteria, and that the small increase was almost certainly due to these organisms; they conclude, that apart from bacteria there is no proteolytic enzyme in milk.

Spolverini([179]) (1902) found evidence of both tryptic and peptic action in all the milks examined by him. The tryptic ferment was rather stronger than the peptic.

Moro([113]) (1902) using Mett's tubes was unable to obtain any satisfactory results. He then used a piece of dried fibrin, suspended it in milk, and estimated the amount of fibrin which was dissolved by the milk. Both faintly alkaline and acid media were used. The experiments were carried out over 12 hours at 37° C. The milk was not known to be bacteria-free. He obtained a solution of ·0009 grammes of fibrin in the acid medium, and of ·0019 grammes in the alkaline medium.

Zaitschek([258]) (1904) used milk with the addition of acid and both with and without pepsin, also with alkali and with and without trypsin. He tested for the formation of peptone, and in no case was he able to demonstrate the presence of these substances unless pepsin or trypsin had been added. The method he found to be correct to ·005 grammes of protein. It is probable, therefore, that Moro's results are within the limit of experimental error. Zaitschek used human, cows', goats' and other milks.

Snyder([174]) (1904) incubated milk and toast at 90° F. for four hours, and found some degree of proteolytic activity, even when chloroform, formalin, or ether was added.

A. J. J. Vandevelde([194, 195, 196]) (1907) working both alone and with De Waele and Sugg, investigated the presence of proteolytic ferments in cows' milk. The milk used was not initially sterile, but was sterilised by means of hydrogen peroxide, or acetone-iodoform solution. The latter he considered most satisfactory, and it was exclusively used by him when working alone. Vandevelde carried out a number of detailed experiments, but no estimation of the amount of protein dissolved appears to have been made under five days' incubation. In most cases the amounts calculated were for 20 days' incubation. The amounts of protein dissolved in five days varied from 2 per cent., which was the more usual amount, to 24 per cent. in one instance. Even, however, if it could be shown conclusively that the action was due to a ferment initially present in the milk, the changes which take place even in five days' incubation are more of academic than of biological interest.

It seemed, therefore, necessary to carry out some experiments with milk which had never been contaminated by bacteria. For this purpose I collected milk by means of a milking-tube, and investigated the presence of a proteolytic ferment. All the experiments gave negative results, and as all were carried out upon identical lines, it will suffice to quote two of them.

The milk was plated out at once upon arrival at the laboratory, and the acidity of a further sample estimated. All the apparatus used was sterilised.

Five flasks were taken and into each of them 50 cc. milk was pipetted. One flask was tested at once, as a control; two others were incubated without further addition, and the remaining two received the addition of as much sterilised solid sodium carbonate as was required to bring the total alkalinity up to ·2 per cent. The experimental flasks were then incubated at 37° C. for from 24-26 hours. They were then removed and the protein precipitated with tannic acid solution, 50 cc. being used for each flask. 25 cc. of the filtrate was then taken and the amount of soluble nitrogen estimated by Kjehldahl's method. The same treatment was carried out upon the control flask without the preliminary incubation.

(i.) *Bacterial count* 23 *per cc.* (*Cow troublesome and kicked out the catheter.*) *Incubation time*, 25 *hours.*

Sample used.	Soluble Nitrogen in cc. $\frac{N}{10}$ solution.
1. Control, 50 cc. milk	3·6
2. 50 cc. milk incubated	3·6
3. 50 cc. milk ,, ...	3·4
4. 50 cc. milk alkali	3·7
5. 50 cc. milk ,, ...	3·7

(ii.) *Bacterial count* 3 *per cc. Incubation time* 26 *hours.*

Sample used.	Soluble Nitrogen in cc. $\frac{N}{10}$ solution.
1. Control, 50 cc. milk	3·75
2. 50 cc. milk incubated	3·7
3. 50 cc. milk ,, ...	3·7
4. 50 cc. milk alkali	3·65
5. 50 cc. milk ,, ...	3·4

It seems, therefore, that initially sterile milk does not contain any ferment acting upon the proteins of milk either in the normal reaction of the milk or in an alkaline medium.

On the Presence of a Proteolytic Ferment in Human Milk.

Spolverini (1902) found both tryptic and peptic ferment action in human milk, and Moro (1902) using the method already described (*see* p. 43), found that ·0006 grammes of fibrin was

dissolved by human milk in 12 hours in the acid solution, and ·0013 grammes in the alkaline sample.

Friedjung and Hecht (1903) using gelatine tubes endeavoured to detect the presence of proteolytic ferments in milk with a view to establishing a relationship between the activity of this ferment and of catalase. The milk was not bacteria-free, and thymol was added. The tubes were incubated for three days, and at the end of that time about 2-3 mm. of gelatine had usually been dissolved; the catalase content differed as much as 8-fold in the sample examined, and the authors conclude that "The proteolytic ferment is not only of too slight activity, but its intensity of action shows too great difference for any relationship to obtain between it and catalase."

Zaitschek (1904) using the method already described (*see* p. 43), was unable to detect any proteolytic action in human milk, of either the peptic or the tryptic variety.

Austin([6]) (1908) working upon the milk of 21 women in different states of health, was quite unable to trace any proteolytic enzyme in human milk.

He used Volhard's method, and the soluble nitrogen was estimated by Kjehldahl's process. No hydrolysis of protein could be detected even after 14-16 days. Austin concludes (1) There is no evidence of auto-digestion of human milk, at least under the conditions pertaining to such digestion in organ tissues. (2) The digestion disturbances of infants fed upon human milk can have no relation to such an enzyme, as the milk of both healthy and sick women was examined.

Summary of the Results upon the Presence of Proteolytic Enzymes in Human and Cows' Milk.

(1) In initially sterile cows' milk there is no proteolytic ferment.

(2) There is no proteolytic ferment present in human milk.

On the Presence of a Peptase in Human and Cows' Milk.

Wohlgemuth and Strich([205]) (1910) working on both human and cows' milk as well as that of other animals, found evidence of a ferment in milk which will hydrolyse glycyl-tryptophane. Warfield([207]) (1911) working upon human milk only, found the same. These authors did not work with initially sterile milk, but treated the milk with antiseptics. In view of this fact, and of the small amount of evidence, the presence of this possible ferment will not be further considered.

On the Presence of "Fibrin-Ferment" in Human and Cows' Milk.

Schlossmann([160]) (1902), Camerer([50]) (1901), and Moro and Hamburger([116]) (1902) found that if human milk was added to hydrocoele fluid, that coagulation took place. Bernheim-Karrer([32]) showed that this was also the case when cows' milk was added to hydrocele fluid. It was supposed that since fibrinogen was

present in hydrocele fluid, that "fibrin-ferment" must be present in the milk. Moro, Camerer (who used blood), and Bernheim-Karrer all found, however, that the reaction was little, if at all, influenced by heat. It is, therefore, extremely doubtful if the substance bringing about this action can be considered to be a ferment. In view of this fact, and of the extremely complicated nature of blood-clotting, this reaction will not be considered any further.

(5) Lipolytic Ferments.

(A) On the Presence of Lipase in Cows' Milk.

Spolverini, Luzzati, and Biolchini (1902), also Marfan and Gillet (1902), found that cows' milk was capable of splitting monobutyrin into butyric acid and glycerine. The action Marfan and Gillet found to be specific for this substance; oil was not acted upon at all, nor other compounds of butyrin.

Moro (1902) found that cows' milk had the power of splitting olive oil. The method used by him is not, however, sufficiently accurate to be convincing. The presence of fatty acid was tested for by the formation of an emulsion when alkali was added.

Leperre([101]) (1904) was unable to trace any decrease in fat content even after several weeks, the other constituents of the milk being, however, altered in quantity.

A. J. J. Vandevelde (1907) determined the acidity of the milk as a test for the formation of fatty acids. The milk was treated with the acetone-iodoform mixture used by him, and after incubation was distilled. No change in the acidity of the distillate could be detected even after several weeks.

On the Presence of Lipase in Human Milk.

Spolverini, Luzzati, and Biolchini, also Marfan and Gillet, all found monobutyrinase in human milk.

Friedjung and Hecht (1903) also found the same, but did not investigate the presence of an autolytic lipase.

Hippius([79]) (1905) using Mankowsky's reagent for the detection of the presence of fatty acids, believed that human milk was capable of splitting olive oil. This reaction was destroyed by heating to 64° C.

None of the investigations connected with the presence of lipase or of monobutyrinase were carried out upon milk collected free from bacteria. This throws the results open to much suspicion. The absence of an autolytic lipase may, however, be assumed to be reliable, and is the only one to which any value need be attributed.

The difficulty of obtaining human milk, and the still greater difficulty of obtaining a reliable method for the investigation of the presence of autolytic lipase, prevented me from investigating the presence or absence of such a ferment in human milk.

The small amount of data upon the presence of mono-butyrinase, and the extremely problematic value of such a ferment to the infant, even if it were found to be present in milk collected free from bacterial contamination, render it unnecessary to dwell any further upon this subject.

(B) On the Presence of a Salol-Splitting Ferment in Cows' Milk and in Human Milk.

The literature of the ferments in milk includes quite a number of papers dealing with the presence in milk of a ferment having the property of splitting salol into phenol and salicylic acid. The presence of this ferment is tested for by adding salol to milk, incubating, and then testing for the presence of phenol and salicylic acid by the addition of ferric chloride.

Nobecourt and Merklen([123]) (1901) first pointed out the presence of this reaction, which was positive in human milk but negative in cows' milk. They also found that it was present in the serum of nursing women, but was absent in the urine.

Spolverini (1901) and Luzzati and Biolchini (1902) also found this ferment present in the milk of women but not of cows.

Moro (1902) showed that no reaction appears under 12 hours' incubation at 38° C. Further, that the reaction is not affected by boiling, which is against its being due to a ferment.

The reaction was positive in all samples of human milk examined by him but always negative in cows' milk.

Desmoulières([54]) (1903) showed that certain salt solutions which are neutral to litmus and phenol-phthalein will split salol in precisely the same manner as the supposed ferment, and that the reaction occurs after boiling; he thinks it must be due to the alkaline phosphates in the milk.

Miele and Willem([111]) (1903) obtained evidence of the presence of salicylic acid in an alkaline solution of salol after incubation for 24 hours at 37° C. They found that the reaction was given by cows' milk and by human milk both raw and boiled, by saliva and by pancreatin, if the reaction of the medium was faintly alkaline.

Desmoulières([54]) (1903) again wrote pointing out that he had already dealt with the question of the reaction of the medium, and had also pointed out the probable sources of error in Nobécourt and Merklen's work.

Friedjung and Hecht (1903) working with human milk found salolase present.

Pozzi-Escot([132]) (1903) showed that the lipase derived from castor oil beans which will attack fatty acid compounds of salicylic acid will not touch the phenol compounds.

A. J. J. Vandevelde([196]) (1907) examined the question of the presence of salolase in cows' milk. He used acetone-iodoform as a disinfectant, and first showed that neither the reagent nor the reaction of the milk was able to produce a splitting of salol. He then took both raw and boiled cows' milk and after adding the disinfectant placed the flasks in the incubator. After 25 days he was able to demonstrate that the raw milk contained salicylic acid of a strength of about ·0001 per cent., and he believed this action was due to a ferment present in the milk.

Grimmer (1910) in his work upon the ferments present in the glands themselves, found salolase present in all the glands examined except in the case of a lactating cow.

Rullmann (1911) using initially sterile cows' milk was quite unable to detect any salolase in the sterile samples examined by him. He concludes therefore that salolase when found in milk is of bacterial origin.

If the results obtained by the various observers be considered for a moment it appears fairly evident that there is no ferment present in cows' milk which can split salol. The only observers who have found it present in any quantity have also found it present in the boiled samples. This is much against the reaction being due to a ferment at all. Nor can any stress be laid upon the minute quantity of salicylic acid produced in Vandevelde's experiments after so long a period as 25 days.

As regards human milk it appears that there may be a body present which is capable of splitting salol, but inasmuch as this action has been found to take place in boiled milk it is doubtful whether it is of ferment nature.

Summary of the Results upon the Presence of Salolase.

1. It appears that cows' milk does not contain any substance which splits salol.
2. Human milk seems to have the power of splitting salol, but it is doubtful whether this action can be attributed to a ferment.

(6).—On the Presence of Lactase and Glycolytic Ferments in Milk.

Spolverini (1902) found evidence of glycolysis present in all the milks examined by him; Zaitschek, however (1904), found no change in reducing power on incubation, except in the case of bacterial contamination, and he hence considered that Spolverini's results must have been due to this cause.

Stocklasa([182]) (1904) prepared a ferment solution from milk by means of alcoholic precipitation, which he considered had lactose-splitting power. The milk used was not initially sterile, but was preserved by means of antiseptics. 50 cc. of a 40 per cent. solution of lactose was used for each experiment, and the amount of lactose lost varied from ·32—·68 grammes. The experiments were carried out over from 3-5 days, at 37° C.

A. J. J. Vandevelde([197]) (1908) believed that he had evidence of the presence in milk of a glycolytic ferment. The milk was not initially sterile, but was disinfected by means of iodoform and acetone. The amount of lactose present was estimated both by the polarimeter and by Fehling's method. There was no increase in reducing power, so that the lactose was not split into dextrose and galactose. The fluids used were found to be bacterial-free as a result of the addition of the disinfecting agent. Vandevelde carried his experiments over very prolonged periods, and the amount of sugar lost, even after many months, was very inconsiderable, and only occurred in the acetone-iodoform samples.

The samples preserved with formol showed no change, or so little as to be negligible. Thus—

—	After 27 days.	After 155 days.	After 429 days.
	Lactose per cent.	Lactose per cent.	Lactose per cent.
Milk sterilised by heat ...	4·63	4·76	4·50
Milk and formol	4·63	4·63	4·31
Milk and acet-idod	4·06	2·22	1·86

In another case, after treatment with antiseptics, there was a decrease of 0·362 per cent. of lactose in 3 years and 5 months.

This work of Stocklasa's and Vandevelde's is open to the objection that the milk was not obtained free from bacterial contamination, but was only rendered sterile afterwards. The varieties of bacteria which will ferment lactose are very numerous, and it is, therefore, not unlikely that traces of ferment action might occur in the solution in presence of the bacteria killed by means of the antiseptics.

The only value that lactase in milk could have for the infant, must consist in the ferment acting with some rapidity upon the lactose within the first few hours. For this purpose the 27 days of Vandevelde's experiment are of no significance, especially in view of the very small amount of sugar decomposed.

It was shown by Aders Plimmer([131]) (1906) that lactase is present in the alimentary canal at birth, and in some animals just before birth, in considerable amount, compared with which the strength of ferment obtained in milk by Stocklasa and Vandevelde, fades into complete insignificance. Various authors have also detected lactase in the alimentary canal of new-born infants (*cp.* Ibrahim). Even if a ferment of the strength found by these observers is present in milk collected free from bacterial contamination, it is difficult to believe that it could have any biological value whatever. For this reason it will not be considered any further.

(7).—Amylase.

The term amylase is applied to a ferment which acts upon starch or amylum, breaking it down into simpler substances, namely, dextrins or sugars.

The fact that starch could be broken down into sugar by the action of ferments has been known for a very long time; as early as 1833 Payen and Persoz prepared a solution from malt extracts which had this power. The substance bringing about this result they termed "diastase."

The work of Fraenckel and Hamburg([65]) in 1906 renders it probable that this substance "diastase" consists of two ferments; one of these liquefies starch, converting it into dextrin, and is hence a true amylase, whilst the other breaks the dextrin down into sugar (maltose), and is, therefore, a "dextrinase." When maltose is further converted into glucose, the action of a third ferment, maltase, is required. (*Cp. also* Duclaux([57]).)

It will be shown in the following pages that the presence of amylase alone appears to have been demonstrated in milk, since most observers have traced the conversion of starch to the dextrin stage only; it may, however, be pointed out that the difficulties of tracing minute quantities of sugar, either maltose or dextrose in milk, is enormous, owing to the presence of the lactose. The evidence of most investigators is in favour of the starch not being split beyond the stage of dextrin.

Amylase in Cows' Milk and Human Milk.

Lépine([102]) (1870) demonstrated the presence of a ferment capable of splitting starch in the large number of tissues examined by him, with the exception of the crystalline lens.

Bécamp([28]) (1882) prepared a ferment solution from milk which had the property of breaking down starch into simpler products. This ferment he called "galactozymase." The action was much stronger in human milk than in cows' milk.

Moro([113, 114, 115]) (1898, 1900 and 1902) was led to the investigation of the presence of amylase by studying the ferment content of infants' stools.

He found that the stools of breast-fed babies contained more diastase than those of babies artificially fed. All the stools showed the presence of the ferment, derived apparently from the alimentary canal, but the stools of the breast-fed babies had a higher content, presumably owing to the presence of the ferment in the milk taken by the mouth.

Moro isolated the protein from human milk with alcohol, and subsequently extracted the precipitate with ether. The residue was dried in vacuo and found to possess definite amylolytic power. Traces of maltose were found, but the starch had for the most part not been hydrolysed beyond the stage of dextrin. In cows' milk, however, the result was negative, using the same method. These results were confirmed by Luzzati and Biochini (1902), who found amylase in human milk and traces of it in asses' milk, but none in cows' milk or goats' milk.

Nobécourt and Sévin([124]) found amylase in the milk of nursing women, and also investigated the presence of amylase in the blood; this will be dealt with later.

Spolverini (1902) found amylase in human and dogs' milk, but was unable to detect any in cows' milk. His well-known feeding experiments will be dealt with later. (*See* p. 56.)

Friedjung and Hecht (1903), working with human milk, found amylase present in all the samples examined by them.

Zaitschek (1904) worked with human and cows' milk, as well as with the milk of other animals, and found evidence of amylolytic ferments in all samples examined. Known quantities of starch were added to the milk, also thymol or toluol, and the samples were incubated for 24 hours.

The amount of starch hydrolysed was estimated by weighing the increase of reducing sugar formed during incubation; the sugar was estimated by weighing the copper oxide after the addition of Fehling's solution. They found increase of copper oxide in all samples, but in varying amounts. The sugar was

calculated as maltose, and 100 cc. cows' milk gave from 0·1-0·4 grammes increase of maltose, whilst human milk gave from 0·1-0·9 grammes increase.

It may be pointed out that these results are somewhat different from those of other observers, in that hydrolysis of the starch was carried a stage further, namely to maltose, instead of to dextrin, as has been found by most observers.

Hippius (1905) also found amylase present in human milk. The ferment will bear a temperature of 65°-69° C., but is inactivated at 70° C.

Koning (1906) in his work upon milk discusses the question of the presence of amylase both in milk and in plants, &c. He showed that certain bacteria were capable of producing amylase, notably B. Mesentericus, B. Subtilis, B. Fluorescens Liquefaciens, and Non-Liquefaciens. He found amylase constantly present in cows' milk which had been carefully collected, and did not think it was due to bacterial contamination. He tried several methods of investigation, and finally used the following:—

10 cc. milk collected with great care were put into a test-tube with a few drops of soluble starch solution, of known strength. This was then incubated, and after varying times iodine (Iodine, 1 part, Potassium Iodide, 2 parts, and water, 300 parts) was added. Small amounts of amylase were always found; about ·015-·02 grammes of starch were decomposed by 100 cc. milk in half hour. Any increase in this amount was pathological. The first and middle milks were richer in amylase than the strippings. The ferment was destroyed by 45 minutes at 68° C.

Giffhorn([67]) (1910) using mixed cows' milk found that 100 grammes of milk will decompose from ·01-·25 grammes of starch. The amount of amylase is increased in pathological conditions of the udder. It is killed by 30 minutes at 65° C.

Wohlgemuth and Strich([205]) (1910) found diastase present in large quantities in human milk, especially in the early days of lactation.

They were unable to detect amylase in either goats' or cows' milk.

Their experiments in regard to the source of the ferment will be returned to later. (*See* p. 56.)

There is, therefore, an accumulation of evidence in favour of the presence of amylase in *human* milk, Koning and Zaitschek alone being able to detect the presence of this ferment in *cows'* milk.

Original Work.

To assist in determining this point, I undertook some experiments upon cows' milk collected by milking-tube, the bacterial count being either nil or negligible.

The method used was that of Koning, and the results were positive on all occasions. It will suffice to quote two experiments since the routine followed was precisely the same in all. The starch solution used was a 1 per cent. solution of soluble starch,

which was made fresh, and sterilised before using. After incubating for the times stated, iodine solution as prescribed by Koning was added to each tube. The boiled controls were always negative, but several controls were always made in order to have varying colour standards for comparison with the ferment-containing tubes, if necessary. In the tubes from which the starch had disappeared a brown colour was struck immediately upon the addition of the iodine, which gradually faded to white, presumably owing to the taking up of the iodine by the protein and other substances present in the milk, which are known to act in this way. The controls gave the characteristic blue iodide-of-starch coloration. The temperature of incubation was 37° C.

The results can be tabulated, and are shown in the following table.

Experiment 1.—*Bacterial count*, 1 *per cc. Incubation time*, 3 *hours at* 37° *C.*

Milk.	Starch solution used =	Grammes of Starch.	Result with Iodine.
10 c.c.	·03 c.c.	·0003	Brown.
,,	·05 ,,	·0005	,,
,,	·1 ,,	·001	,,
,,	·2 ,,	·002	,,
,,	·3 ,,	·003	Blue-gray.
,, (boiled)	·03 ,,	·003	Blue.
,, ,,	·1 ,,	·001	,,

Experiment 2.—*Bacterial count*, 3 *per cc. Incubation time*, $3\frac{1}{2}$ hours at 37° *C.*

Milk.	Starch solution used =	Grammes of Starch.	Result with Iodine.
10 c.c.	·1 c.c.	·001	Brown.
,,	·2 ,,	·002	Faintly gray blue.
,,	·3 ,,	·003	Blue.
,,	·4 ,,	·004	,,
,, (boiled)	·1 ,,	·001	,,
,, ,,	·2 ,,	·002	,,

It seems, therefore, certain that cows' milk does contain small quantities of amylase, 10 cc. milk being able to split from ·001-·002 grammes of starch, in 3 hours at 37° C.

Summary of the Work on Amylase in Cows' Milk and Human Milk.

The evidence shows that—

(1) Amylase is present in human milk, in all the cases examined.

(2) That it is present in small quantities in cows' milk, but has frequently not been detected.

(3) The ferment hydrolyses starch to dextrin, and is hence a true amylase.

Division B.—Summary of the Results Obtained in Division A.

The work dealt with in the preceding sections shows that—

1. Peroxidase is constantly present in cows' milk, but is very inconstant in its appearance in human milk. The reaction for the detection of this ferment is, on the whole, more marked in colostrum, or in mastitis milk. (*cp.* Weber, Giffhorn, Marfan and Gillet, Friedjung and Hecht, and Kastle and Porch.)
2. The ferment comes down with the albumin fraction; it is most probably derived from broken-down cell-tissue, in the case of human milk probably from the leucocytes.
3. The reaction is believed to be dependent for its production upon the presence of a metallic compound (iron or manganese) in colloidal state; it is destroyed by acid, by any excess of alkali, or by digestion.
4. Catalase is present in small amounts in milk collected initially free from bacteria both in cows' milk and in human milk.

   The amount is increased in colostrum or in mastitis milk, or, in women's milk from badly acting glands.
5. In ordinary milk the major part of the catalase is derived from the contained bacteria.
6. Direct reductase is derived exclusively from the contained bacteria in milk, whether cows' milk or human milk.
7. Indirect reductase, or formaldehydase, is present in sterile cows' milk, which has never been contaminated by bacteria; it is most frequently absent in colostrum. It has some connection with the fat of the milk.
8. Indirect reductase is absent in human milk.
9. The hydrogenase said to have been found in cows' milk is of bacterial origin.
10. Proteolytic ferments are not present in milk collected free from bacteria in either cows' or human milk. A peptase has been described in milk not known to be sterile, but this has not hitherto been confirmed.
11. Lipase is not present in cows' milk. A ferment, monobutyrinase, is stated to be present in both human and cows' milk, the milk not being known to be initially sterile.
12. Salolase has not been found in cows' milk collected free from bacteria; it is stated to be constantly present in human milk (bacterial content not given).
13. Traces of lactase and of glycolytic ferment have been found in cows' milk not collected free from bacterial contamination.
14. Amylase is present in small amounts in cows' milk collected free from bacteria. It is also constantly present in human milk.

The results obtained in the preceding part of this work may be tabulated as follows:—

Name of Ferment.	Presence in pure Cows' Milk.	Presence in pure Human Milk.	Of Bacterial Origin.
Peroxidase	Constantly present	Inconstant	—
Catalase	Present	Present	In great part
Reductase—direct ...	Absent	Absent	Yes
Reductase—indirect ... (formaldehydase).	Present	Absent	No
Hydrogenase... ...	Absent	No data	Yes
Proteolytic	Absent	Absent	Yes
Peptase	Present (?)	Present (?)	(?)
Lipase	Absent	No data	—
Monobutyrinase ...	Present (?)	Present (?)	(?)
Salolase	Absent	Present (?)	(?)
Lactase and glycolytic	(?)	No data	—
Amylase	Present	Present	—

The ferments known to be present in pure cows' milk are catalase, peroxidase, formaldehydase, and amylase.

Those known to be present in human milk are catalase, amylase, sometimes peroxidase, and possibly salolase, although this has not been investigated in milk known to have been sterile.

In the case of the others mentioned the evidence is scanty, and the work has not been carried out in initially sterile milk.

Division C.—ON THE BIOLOGICAL VALUE OF THE FERMENTS PRESENT IN COWS' MILK AND IN HUMAN MILK, IN THE FEEDING OF INFANTS.

It has been shown in the preceding part of this report that the ferments which are present in cows' milk, apart from the action of the contained bacteria are, catalase, peroxidase, formaldehydase, and amylase.

Further monobutyrinase, glycyl-tryptophanase, and glycolytic ferments have been found in milk not initially sterile.

Those ferments which are found in pure human milk are catalase, amylase, and sometimes peroxidase; whilst salolase, monobutyrinase, glycyl-tryptophanase have been found in milk not known to be initially sterile.

These ferments can be grouped under three headings:—

1. Those acting upon substances present in the milk, and presumably concerned in digestion.
2. Those acting upon substances not known to be normally present in milk.
3. Those concerned with oxidising or reducing processes.

Under the *first* heading the trace of lactase found in milk not initially sterile alone appears, and as this subject has already been dealt with in some detail, it need not be considered any further here. (*See* p. 47.)

Under the *second* heading appear amylase, monobutyrinase, glycyl-tryptophanase, and the salolase in human milk. These last three ferments have not been shown to be present in initially sterile milk; the literature of the ferments shows that ferments are specific in their action, and there is no reason to suppose that these ferments (if present in uncontaminated milk) would act upon any other substances than those from which they derive their names. As these substances are not known to be present in either human or cows' milk, it is hardly possible to suppose that the presence in milk of the ferments which act upon them, can have any biological significance.

Formaldehydase also strictly comes under this heading. It is not, however, at all certain that this action is dependent for its production upon the presence of a ferment, since it has been shown that it can be restored after removal by heat by either alkali or ferrous sulphate. As, however, it is entirely absent in human milk, it may reasonably be assumed that it is without biological value to the infant.

Amylase remains to be considered. It is present in cows' milk in amounts too small to split any appreciable quantity of starch, but is stated to be present in somewhat larger amounts in human milk. Clinical experience has definitely shown that starch is an unsuitable food for infants during the first few months of life. Further, it has been shown that amylase is present in the saliva and pancreatic juice of new-born infants (*cp.* Zweifel([210]), Moro([114]), Ibrahim([81])), so that the amylase of the milk is not in any case required for the digestion of starch, even were such digestion necessary in the early months. It is not necessary to seek further for any possible function for the amylase of milk.

Under the *third* and last heading catalase and peroxidase remain to be considered. It has been shown that catalase is present in small amounts in all tissue extracts, so that its absence in milk, rather than its presence, would be cause for inquiry. As a whole the healthier the gland the less the catalase, the amount being greater in colostrum or mastitis-milk.

The irregularity of the appearance of peroxidase in human milk would alone be a strong argument against attributing any value to this ferment in the general nutrition of the infant. Peroxidase is, moreover, destroyed by acid, and by digestion, so that its life as a ferment must be ended on reaching the stomach.

Clinical evidence has also shown that no difference can be detected between infants, the milk of whose mothers contains varying amounts of these ferments, or even no peroxidase.

Peroxidase, catalase, and amylase are found in considerable amounts in the blood, and it is only consonant with general physiological experience to suppose that the small and varying amounts of these ferments which are found in milk are derived from the blood-stream by filtration.

The presence of amylase in the blood has been known for many years, and was found by Achard and Clerc([1]) (1901) to decrease

in certain disease (*cp. also* Lepine[102] and Bial[36]). Nobécourt and Sévin[125, 126] (1902) showed that the amylase content of the serum of wet-nurses and of cows varied very considerably. Amylase was present in both urine and milk of the wet-nurses, and in the urine of cows, but they failed to detect it in cows' milk, presumably owing to the small amount present. The amylase content of the urine was always higher than that of the milk. They were unable to detect any difference in the amylase content of the blood of breast-fed and artificially-fed infants.

The presence of catalase and peroxidase in the blood is so generally known that no evidence need be adduced in this connection. Senter[169], Kastle and Amoss[85] v. Itallie[82] and many others may be compared.

It appears that the ferment content of the blood can be altered by various conditions. Thus Dubourg[55] (1889) found that both in himself and in rabbits the amylase content of the urine was increased on a diet rich in starch.

Spolverini[179, 180] (1902) fed lactating animals upon food containing ferments other than those normally present in the milk of that species, and obtained evidence of the presence of these foreign ferments in the milk.

Landtsheer and Vandevelde[98] (1903) did not confirm Spolverini's results, but Spolverini wrote pointing out that his procedure had not been followed, and that the criticisms were not justified. (Arch. de méd. des Enfants, 1904.)

Wohlgemuth and Strich[205] (1910) tied the pancreatic duct of a bitch in full lactation, and found that the amylase content of blood and urine, and subsequently of the milk, increased. The blood content was always higher than that of the milk, but that both fell together.

It seems that ferments can pass from the blood into the milk, and that this can account for the presence in the milk of ferments identical with those in the blood, more especially as the amount of these ferments tends to increase when, owing to disease of the gland, and inflammation, the exudation of serum is increased in amount. Some portion of the ferment content is, however, probably derived from the leucocytes of the milk and from the breaking-down of the cells of the gland itself in the course of its metabolism.

As a result of the survey of the available data upon the ferments present both in human and in cows' milk, there appears to be no ground for claiming any biological value for these substances.

PART III.

ON THE SO-CALLED "PROTECTIVE SUBSTANCES" IN MILK.

In this part of the report no attempt is made to deal fully with the whole question of the protective substances in milk. The field has been purposely strictly narrowed to include only those substances which have been shown to be present either by direct investigation, or by feeding. Any attempt to deal with the biology of milk in relation to the antigens, whose nature is determined by injection, leads at once into the most subtle and complex branches of biological research. This has purposely been omitted, so as to concentrate as closely as possible upon the bearing of the question on the feeding of infants. Further such side issues as necessarily arise have been treated very briefly, without attempting to discuss the literature, again in the endeavour to avoid complication of the main issue.

The term "protective substance" has been applied to certain bodies in the blood which have been shown by experiment and subsequent practice to afford protection, and even immunity, to the organism from certain diseases. It does not, however, necessarily follow, nor can it be assumed *a priori* that, should these substances be found in milk, they subserve the same function as when present in the blood-stream.

No original work has been carried out for this part of the report, and no attempt will be made to deal with the basis of our present knowledge of the bodies concerned in the production of this immunity; an elementary acquaintance with the fundamentals of this branch of knowledge will be assumed. The presence of such bodies, and their possible value to the infant as far as is known from the work at present published will be considered.

Division A.—On the Presence of "Protective Substances" in Milk.

These substances may for the present purpose be conveniently considered under five headings according to their known action.

1. Those concerned with hæmolytic action.
2. Those concerned with bactericidal action.
3. Those bringing about the precipitation of certain substances (precipitins).
4. Those bringing about agglutination of bacteria (agglutinins).
5. Antitoxins.

1.—*The substances concerned in hoemolytic action.*

Three bodies are necessary for hæmolysis to occur; the red corpuscle, the amboceptor or immune body, and complement. Further for such a series of bodies to form a hæmolytic system

the red corpuscles used must be such that the amboceptor is capable of sensitising the red corpuscle and thereby rendering it susceptible to the action of the complement. Each variety of corpuscles requires its own amboceptor, which is specific for that particular variety, and it is therefore evident that there are a large number of bodies functioning as hœmolytic amboceptors for the blood of different animals.

It has now to be considered whether there is evidence of the presence of either amboceptor or complement or both, in milk, and cows' milk will be dealt with first.

The hœmolytic factors of cows' milk.

If cows' milk contained both amboceptor and complement hœmolysis would occur on the addition to the milk of a suspension of suitable red corpuscles. This however has not been found to occur by any observer using average milk. In 1907 Pfaundler and Moro([274]) obtained evidence of the presence of complement in cows' milk by using the system.

Guinea-pigs corpuscles (·2 c.c. 5 per cent.) + inactive ox-serum (·05 c.c.).
+ raw milk (·5 c.c.).

The system guinea-pigs corpuscles + inactive ox-serum + ox complement being a suitable one. Using the same system in the following year I([256]) also obtained evidence of the presence of complement in cows' milk. The subject was discussed at a meeting of German pediatricians, when Noeggerath and Bauer both stated that they had been unable to obtain hœmolysis after the method of Pfaundler and Moro, except in colostrum or mastitis-milk. Noeggerath([273]), however, subsequently wrote saying that Moro had demonstrated to his and Bauer's satisfaction that hœmolysis could be obtained in milk from a healthy cow but that the amount was very small and the demonstration of its presence depended upon the relative amounts of the serum and milk which were used.

In the same year as the discussion, Kopf([251]), working in Schlossmann's laboratory under Bauer, was unable to trace any complement in cow's milk using the same system as Pfaundler and Moro, and myself, except in colostrum, in which complement could be readily demonstrated.

Sassenhagen and Bauer([217]) (1909), showed further, that complement appeared in easily demonstrable quantities in mastitis-milk, and that early mastitis could be detected by this test before the fact could be diagnosed clinically.

Sassenhagen([290]) (1910) showed that complement is present in considerable amounts in the colostrum, both of cows' and goats, and that it depends for its appearance upon the colostral character of the milk, disappearing gradually although somewhat irregularly, as the milk assumes the normal character.

Moro and Pfaundler([274]) failed to obtain evidence of the presence of amboceptor in milk.

In 1908 I believed that I had obtained evidence of the presence of hoemolytic amboceptor in cows' milk. By incubating a mixture of heated ox-serum and guinea-pigs corpuscles a solution (in which, after removal of the corpuscles, I was unable to detect the presence of amboceptor) was obtained and which on being added to milk in the presence of guinea-pig's corpuscles and fresh serum gave hoemolysis. Kopf however states that in his experiments the amboceptor did not become attached to the corpuscles on digestion unless complement were also present. Dr. Bauer, under whom Kopf carried out his work, has since been good enough to show me some figures of his own which bear out his view. Since seeing these figures, this spring, I have not had an opportunity of re-investigating the matter myself.

Both Kopf and Sassenhagen found amboceptor present in colostrum, during the first few days after parturition, hoemolysis occurring with guinea-pigs corpuscles and milk, alone, and the same was found by me in the case of milk from a cow a few days after parturition.

It appears therefore that both amboceptor and complement are present in the early colostrum (the complement persisting until the complete disappearance of the colostral characters), and possibly afterwards in small quantities, although this is a matter upon which authors are not entirely agreed.

Human milk.

Cattaneo([223]) (1905) believed that he had obtained evidence of complement in human milk, using human corpuscles. In 1907, however, Frey([232]) pointed out that in Cattaneo's experiments there were traces of serum left with the corpuscle which should be removed by washing, and that if this is done no hoemolysis is obtained. Further that Cattaneo's asepsis was not complete, and the experiments were carried out over sufficient length of time(36 hours) to introduce the possibility of bacterial action.

Pfaundler and Moro([274]) (1907) were unable to obtain any evidence of the presence of complement in human milk, using the same system as for cows' milk except for the different milk, that is g. pig r.c. ·5 c.c. + inactive ox-serum ·05 c.c. + human milk ·5 c.c. and also rabbits r.c. ·5 c.c. + human inactive serum ·25 + human milk ·5 c.c.

They believed that there was an inhibitory effect when large amounts of milk were used because, if the following mixtures were made and incubated (*a*) 0·5 cc. g. pig r.c. + ·15 human inactive serum + 5 c.c. human milk, and (*b*) 0·5 cc. g. pig r.c. + ·15 human inactive serum + 5 cc. saline and after two hours incubation, 0·5 cc. active human serum was added to both, the result was incomplete hœmolysis in (*a*) and complete hœmolysis in (*b*). They believed this to be due to some alteration of the red corpuscles which rendered them less easily attacked by the hœmolytic substances.

Bauer([215]) (1908) showed that the inhibitory effect obtained by Moro and Pfaundler was not due to alteration in the corpuscles, since if the corpuscles were washed after the first incubation, there was no inhibition of hoemolysis. But he believed that there was

an inhibitory effect, because if human milk was added to a complete hœmolytic system inhibition of hœmolysis occurred if the milk was added in sufficient amounts. Decreasing amounts of human milk gave increasing amounts of hœmolysis.

In 1908 Pfaundler and Moro([276]) showed that the presence of complement in human milk could be demonstrated, if the serum of a rabbit immunised against sheep's corpuscles was used, thus— 0·1 cc. 1 per cent. sheep r.c. + 0·1 cc. immune serum (dil) + 0·2 cc. human milk. With this system they obtained hœmolysis, and hence evidence of the presence of complement in human milk.

Noeggerath and Kolff([272]) (1909) used a variety of suitable systems, and with the exception of the merest traces on a few occasions, were unable to detect the presence of any complement in human milk. On using the method of Pfaundler and Moro, however, and also other immune sera, they obtained hœmolysis in some cases, and thus demonstrated the presence of complement in human milk.

They did not consider that any real inhibitory effect could be postulated since this only occurs when there is previous incubation of the systems used (cp. Pfaundler and Moro) before the addition of the active serum. By this procedure marked inhibition could be obtained with human milk. If previous incubation of the mixture were not carried out the inhibitory effect could only be demonstrated when comparatively large amounts were used. The same was also found by Moro and Pfaundler.

It appears therefore that complement in human milk can only be demonstrated when an extremely sensitive system is used, which means that it must be present in very small amounts.

Before leaving the question of the hœmolytic factors in milk the work of Bulloch and of Kraus should be mentioned.

Kraus([254]) (1901) immunised lactating goats to sheep's corpuscles, and found hœmolytic power in their blood, but the addition of sheep's corpuscles to their milk, did not bring about hœmolysis. Kraus concludes that the immune body did not pass out in the milk (*cp.* Famulener, p. 72).

Bulloch([222]) (1902) immunised female rabbits to ox corpuscles, and showed that the milk from these rabbits, which were shortly afterwards in lactation, contained the specific immune body, as was shown by the fact that the addition of complement to the milk, together with ox corpuscles, gave hœmolysis.

Kraus does not appear to have added any complement, which may account for his negative results.

The blood of an actively immune animal is much more sensitive than the blood of one not specifically immune, and this may account for the difference of results obtained between the work upon amboceptor in normal milk, and in that from an immune animal. Normally there is not enough amboceptor present to be detected; it is difficult to believe that traces may not be present.

Summary of Section 1 :—

1. Colostrum contains both complement and amboceptor. Amboceptor is only demonstrable during the first few days after parturition, whereas the complement persists longer.

2. The evidence as to the presence of complement in both human and cows' milk after the colostral period is somewhat conflicting. If detected it is found only in quite small quantities.

2.—*The Presence of Bactericidal Substances.*

The factors concerned in bacteriolysis are on parallel lines with those concerned in hœmolysis, the difference being that the amboceptor must be capable of being attached to the bacteria instead of to the red corpuscles, the complement functioning in a similar manner as in hœmolysis.

The amboceptor is specific for different varieties of bacteria, just as it is for different varieties of red corpuscles. Hence in dealing with the bactericidal properties the species of bacteria must be given. In dealing with milk the difficulty is increased owing to the bacteria already present in this fluid, unless extra precautions be taken in milking and later. For this reason some observers have studied the general effect of milk upon the life of the bacterial flora usually present in milk, and others, taking milk freed from the usual flora, have studied the specific action of milk upon different varieties of bacteria.

The importance of ascertaining the viability of bacteria in milk extends much beyond the field of infant feeding, being of great importance in the question of the transmission of disease. In this connection the viability of B. Typhosus and B. Choleræ have been studied especially by the earlier investigators, who were anxious rather to establish the existence or otherwise of the organism than to discover whether there was any decrease in the numbers. Later workers have studied the number of the organisms present in much greater details, with a view to the detection of amboceptors and complement whose presence might perhaps be of value from the biological aspect.

The investigations, as a whole, showed that B. Choleræ died in milk as soon as the milk became sour. (*cp.* Wolffhugel and Riedel([298]), Kitasato([253]), Heim([243]), Uffelmann([294]), Friedreich([233]), Basenau ([213]), and others), whilst Weigmann([297]) and Hesse([244]) both believed that the organisms were destroyed in a few hours by virtue of the inherent bactericidal power of milk. Fokker([234]) also obtained an initial decrease.

Schottelius([291]) (1896) using B. Diphtheriæ found that raw milk was a much better medium for this organism than either sterilised milk or broth, growth being more rapid in the raw milk.

Conn and Ester([225]) (1901) studied the growth of varieties of bacteria at 20° C. The bacterial count was made at the time of the inoculation, and as a rule six hours later. They found that B. Acidi Lactici increased in numbers from the first, although the total numbers of bacteria present remained fairly constant, owing presumably, to the death of some of the varieties of bacteria for whom milk was an unsuitable medium. Streptococci also tended to show an increase per cent. of the whole bacterial flora present, but there was sometimes a slight absolute initial decrease, during

the first six hours. Some organisms which could not be detected in the first estimation, subsequently appeared rather suddenly in the subsequent observations.

Cozzolino([227]) (1902) used cows', goats', asses' and human milk. The milk was carefully collected and was then heated on eight successive days to 55-58° C. for one hour. The milk thus treated, was inoculated with B. Coli, and was not found to be a good medium. Of the varieties of milk, human milk showed least growth, an inhibition and even a decrease occurring.

Moro([261]) (1901 and 1902) investigated the presence of definite bacteriolytic factors in milk, but was unable to trace the presence of any such bodies in either human or cows' milk.

Klimmer([248]) (1903) was unable to detect any bactericidal effect in either asses', cows' or human milk towards B. Coli or B. Typhosus.

Conn and Stocking([224]) (1902) did a good deal of work upon the relative numbers of the organisms forming the ordinary bacterial flora of milk. They found that there was nearly always a decrease in the total number of bacteria during the first few hours after milking. They then investigated the changes in the various groups of organisms present, notably those of the acid-forming group. The results could be classified into three groups:—

1. Those in which there was a decrease in the total number of organisms during the first three hours; this was accompanied by a rapid increase in the number of the acid-forming bacteria, not only absolutely, but also in per cent. of the total.
2. A decrease in the total number including the acid-forming ones. This occurred when there were many varieties of acid-forming bacteria present.
3. A slight initial decrease followed by rapid increase, B. Acidi Lactici grew rapidly at first, but afterwards more slowly, decreasing after the first six hours in actual percentage, owing to the still more rapid growth of non-acid-forming varities.

The following table which shows an experiment falling into group 1, gives a good idea of the general trend of the data given.

Time after Milking.	Total Bacteria per c.c.	Acid-forming per c.c.	Liquefying per c.c.	Per cent. of Acid-forming.
Fresh	12,550	1,250	200	10
Three hours ...	12,250	2,000	200	16
Six hours	19,650	2,250	800	23
Nine hours ...	56,900	20,250	550	36
Twelve hours ...	114,250	68,400 (Practically all B.A. Lact.)	1,900	60

P. Sommerfield([293]) (1905) put milk through a porcelain filter, and used the sterile serum thus obtained to investigate the bactericidal properties of the milk. He found no bactericidal power towards B. Coli or B. Typhosus.

Kolle([250]) (1905) using fairly germ-free milk obtained some evidence of the inhibtion of growth of B. Choleræ but not of B. Coli or of B. Typhosus.

Koning([252]) (1905) carried out experiments upon the numbers of bacteria present in the same sample of milk directly after milking and later. He found as other observers had done, that there was an initial decrease in the total number of bacteria. This decrease was most marked in milk which had been collected as far as possible free from bacterial contamination, being less marked in dirty milk. The optimum temperature was 37° C. The phenomenon was absent in milk which had been boiled, but was retained for a longer period if the milk was kept cool. Koning believed that milk contained substances inhibitory to the growth of bacteria, which were probably derived from the blood.

Rullman and Trommsdorff([285]) (1906) also found that the inhibitory effect upon the ordinary bacterial flora was increased in carefully-collected milk, and lasted almost throughout the first day, even at room temperature. This property, together with the leucocyte count was increased in mastitis.

St. John and Pennington([281]) (1907) found that bacteria grew more readily in pasteurised milk than in raw milk, and that the acid-forming organisms in particular grew much more rapidly.

Moro([264]) (1907) re-investigated the action of raw cows' milk upon typhoid bacilli, and found that raw milk had a slight action, but that this was absent in boiled milk or in the serum obtained by putting milk through a porcelain filter. The inhibition was not great, nor was the growth in the boiled samples always large. The inhibitory effect was almost entirely destroyed by heating to 56° C. for half-an-hour.

The following figures illustrate the results obtained by Moro.

———	Hours after Milking.			
	0.	1.	3.	7.
Bacteria per c.c. in raw milk	466	357	258	4,000
Bacteria per c.c. in boiled milk	222	285	1,960	Infinite.

Moro discusses the literature and points out that the different results obtained by the various observers, means that there is no marked action, owing to the small amount of the bactericidal substance present.

Myer Coplans([226]) (1907) inoculated raw milk and boiled milk (heated to 100° C. for one hour on two successive days) with cultures of B. Coli. He obtained slight bacteriolysis and inhibition of growth for six hours after milking at 20° C., and partial inhibition for some hours more. At blood-heat the same inhibition only lasted for one hour, and the partial inhibition for from 2-6 hours after milking. At 0° C. the inhibition lasted for 24 hours. The rate of growth in boiled milk was taken as the standard of growth without inhibition. He points out that infants fed upon shop milk can obtain little benefit from this power of milk.

Rosenau and McCoy([283]) (1908) studied the action of milk on bacteria. They showed that the apparent bactericidal effect which occurred in fresh milk was really due to agglutination of the bacteria, and could be shown to be entirely absent if the agglutinated masses of bacteria were broken up before plating out.

This decrease occurred only in the first 6-8 hours after milking, and was not found in boiled milk or in milk heated to 80° C. It was not effected by freezing, but was hindered by dilution. The action was specific, since it was not the same for different species of bacteria, nor was it the same in different milks, and even in different samples from the same animal. They also showed that the agglutinating and therefore the inhibitory effect could be apparently restored by adding a drop of typhoid agglutinating serum, as shown in the table below.

They also showed that the action was not due to leucocytes, since it was present in milk which had had the corpuscles removed by centrifuging. The breaking up of the clumps was accomplished by drawing the milk up and down a fine pipette pressed against the bottom of the vessel.

The milk was almost germ-free, and the samples after inoculation were kept at 37° C.

—	Bacteria per Loopful.					
	At once.	2½ Hours.	4½ Hours.	6½ Hours.	8½ Hours.	
					moderate shaking.	mixed with pipette.
B. Typhosus raw milk ...	1,880	1,380	1,060	1,480	1,980	12,200
Do. boiled milk ...	2,120	4,020	*a. b.* 800,000	*a.*	*a.*	*a.*
Do. one drop typhoid serum added.	2,100	2,040	1,920	2,300	1,260	*a. b.* 20,000
Do. boiled	2,280	2,360	7,020	6,480	10,860	*a. b.* 60,000
B. Typhosus in broth, serum added.	1,830	970	2,920	9,180	11,160	*a. b.* 100,000
Organisms in milk alone ...	0	1	2	2	2	46

a. = Innumerable. *b.* = About.

This table is most interesting and shows the effect due to agglutination.

Much([269]) (1908) showed that milk treated with hydrogen peroxide retains its apparent bactericidal properties much longer than ordinary milk. If however the hydrogen peroxide be added as late 20 hours after milking, then it has no influence, the normal apparently inhibitory effect having worn off before the addition of the disinfectant.

Bartelli([212]) (1909) believed that the decrease in the ordinary bacterial flora was due to the gradual development of acidity in the milk owing to the growth of lactic acid organisms. If the milk was kept cool during the period of usual inhibition then the acidity did not rise and there was no inhibition.

In the following table he showed that the inhibition starts when the milk is kept at the optimum temperature for the development of the lactic acid organisms, namely 37° C.

Time after inoculation.	Milk I (kept at 0° C.).		Milk II (at 0° C. for 6 hours, then at 37° C.).		Milk III (at 37° C.).	
	Bact. per c.c.	Lactic acid per cent.	Bact. per c.c.	Lactic acid per cent.	Bact. per c.c.	Lactic acid per cent.
At once ...	143,000	0·0011	143,000	0·0011	143,000	0·0011
Three hours ...	145,000	0·0015	145,000	0·0015	140,300	0·0133
Six hours ...	145,600	0·0016	145,600	0·0016	129,000	0·1226
Nine hours ...	149,000	0·0018	141,250	0·0106	190,000	1·0037
Twelve hours .	151,000	0·0025	127,000	0·0762	253,000	1·1009

The inhibition shown by these figures cannot be considered to be other than very small.

Sassenhagen([290]) (1910) working with colostrum obtained evidence of bactericidal effect upon B. Coli, which was much stronger than in ordinary milk.

Bub([221]) (1910) carried out numerous experiments upon the action of colostrum upon various organisms. He first of all tested the effect upon the ordinary flora of milk collected without any special precautions. He obtained evidence of some inhibition at 15-18° C. and less at 37° C., viz. :—

Time after Milking.	Bacteria per c.c. at 15–18° C.	Bacteria per c.c. at 37° C.
At once	783	848
Two hours	604	754
Four hours	716	940
Six hours	818	5,800
Eight hours	739	26,340
Ten hours	636	70,000
Twelve hours	1003	150,000

Bub then took milk collected with all possible precautions and inoculated it with various organisms, B. Coli, B. Paratyphosus, and B. Pyocyaneus, and obtained a well marked decrease in the bacterial count of the samples inoculated with each of the organisms, the effect wearing off in a shorter time at 37° C. than at 15-18° C., viz. :—

Time after inoculation.	Count of B. Coli.		Count of B. Paratyp.		Count of B. Pyocyaneus.	
	At 15–18° C.	At 37° C.	At 15–18° C.	At 37° C.	At 15–18° C.	At 37° C.
At once ...	3,345	3,648	3,582	3,453	13,425	12,785
In two hours	1,372	711	—	2,952	11,520	13,544
In four hours..	887	2,160	3,489	2,840	7,485	18,870
In six hours...	872	27,000	—	8,570	6,892	30,000
In eight hours	845	70,000	3,172	19,470	22,680	Infinite.

If the tubes were well shaken before taking the bacterial count no inhibition was detected although the count is much higher in boiled milk than in raw.

—	No. at once after inoculation.	No. after 6 Hours. (Moderate shaking.)	No. after 6 Hours. (Strong shaking.)
B. Coli in raw milk ...	1,184	8,120	49,000
Do. sterilised... ...	1,145	2,000,000	—
B. Parat. in raw milk ...	6,342	3,873	57,000
Do. sterilised ...	6,480	Infinite.	—
B. Pyocyaneus in raw milk	11,870	17,760	Infinite.
Do. sterilised	15,084	Infinite.	—

There seems therefore little doubt as to the important part played by agglutination. The further fact that the growth is less rapid in raw milk than in boiled, does not necessarily postulate any bactericidal or inhibitory effect as compared with the rate of growth in sterilised milk, because as has been pointed out by various authors, this may merely be due to the fact that the chemical changes which take place in prolonged boiling or sterilising may render the milk a better medium, and growth proceeds at a more rapid rate.

Gutzeit([237]) (1911) studied the bacterial count in filtered milk, and did not think that the count increased any more rapidly in the filtered milk than in the control milk; he points out that allowance must be made for the length of time which elapses in the process of filtering, the temperature, &c.

Kleinschmidt([247]) (1911) examined both human and cows' milk for the presence of bacteriolytic amboceptor for B. Coli and B. Typhosus. He believed that he had obtained evidence of small amounts of these bodies in both forms of milk. He took carefully collected milk which was almost germ-free. This was inoculated with cultures of B. Coli and B. Typhosus, the amount of the inoculation being determined by plating out. He endeavoured to avoid agglutination effects by shaking, but details are not given. Kleinschmidt concludes from his figures that amboceptor for both Coli and Typhosus are present in cows' milk. In the colostrum of a cow two days post-partum he found much stronger effect, and considers that complement is present as well. The figures given below are typical of the results obtained in various experiments.

Number of bacilli inoculated per cc. were B. Typhosus 1,720, and B. Coli 3,000.

—	Typhoid after 3 hours.	Coli after 3 hours.	Coli after 7 hours.
Raw milk	128	2,800	30,000
Milk heated to 56° C. ...	2,840	6,480	Infinite.
Do. + ·01 c.c. human serum	108	364	50,000
Milk heated to 100° C. + ·01 human serum.	10,000	50,000	Infinite.

He thinks that bacteria probably grow quicker in boiled milk than in raw, apart from the possible destruction of bactericidal substances.

The work upon the bactericidal substances in *human* milk must now be considered; it is neither large nor very definite.

Honigmann([245]) (1893) inoculated human milk with B. Typhosus, B. Coli, and Staphylococcus Aureus. He obtained some inhibition of growth but no bactericidal power within the first 6½ hours after milking. The inhibition he believed to be due to the protein, since if blood was diluted until the percentage of protein was that of the milk only an occasional decrease was noticed, and frequently no action at all.

Moro([261]) (1901) failed to obtain any trace of bactericidal substance in human milk, whilst Cozzolino([227]) (1902) found that there was an initial inhibition of growth, and even a slight decrease in number.

Klimmer([248]) (1903) was able to demonstrate that in human milk there is occasionally a slight inhibitory effect upon B. Coli and B. Typhosus in the first few hours.

Moro([264]) in 1907 believed that he had obtained evidence of traces of bactericidal action upon typhoid bacilli, but Noeggerath and Kolff([272]) (1909) criticised his results as not really showing any effect and they themselves were unable to demonstrate any. They found, as did Moro, that boiled milk, and milk put through a Berkefield filter did not give any inhibition, and agreed with Moro that boiled milk probably provides a better medium for growth than raw milk. It is not necessary to enter into a detailed discussion of the results obtained by these observers. Probably traces are present but it may be that the amount is frequently too small to be demonstrable, and varies in the different samples of milk.

Kleinschmidt([247]) (1911) using the same procedure as for cows' milk worked with human milk and human colostrum, and believed that he had obtained evidence of the presence of amboceptor for both B. Coli and B. Typhosus, and thinks that it may be of use to the infant if it gets into the blood (*cp.* below, p. 69 *et seq.*).

He gives the following figures:—

—	B. Coli. Inoculation 3,600 per c.c.		B. Typhosus. Inoculation 2,320 per c.c.			
			Milk.		Colostrum.	
	After 3 hours.	After 7 hours.	After 3 hours.	After 7 hours.	After 3 hours.	After 7 hours.
Raw milk approx. ...	30,000	Infinite.	5,630	10,000	3,840	10,000
Heated to 56° C. ...	50,000	Do.	5,800	20,000	5,600	10,000
Do. + ·01 human serum.	3,260	20,000	44	1,040	1,160	4,400
100° C. + ·01 human serum	50,000	Infinite.	5,780	Infinite.	6,340	Infinite.

If it be admitted that boiled milk is a better medium for the growth of these organisms, then the figures do not appear very conclusive.

3.—*The presence of precipitins.*

When a foreign protein gains entrance into the blood-stream of any animal, it evokes a reaction in the tissues of the animal, a substance being produced which is capable of forming a precipitate with that foreign protein and thus throwing it out of action.

Such bodies which are capable of throwing out foreign proteins are known as precipitins. The reaction is an exceedingly delicate one, and very small amounts of foreign protein can be detected.

This reaction has, as will be seen later, been largely used in connection with the absorption of protein from the alimentary canal.

Precipitins are not normally present in serum, so that unless under exceptional circumstances they are not to be expected in the milk. Langer([257]) (1907) however showed that the antigens or substances which can produce precipitins in the blood of another species pass out into the colostrum in considerable amount, and that cows' colostrum has a much higher content of these substances than later milk.

4.—*The presence of agglutinins in milk.*

It has already been shown that milk has agglutinating power in dealing with the bactericidal action of this fluid, and it will not be necessary to dwell much longer upon this point. Kraus([254]) (1901) found that artificial agglutinins if present in the blood were passed out into the milk and Langer([257]) (1907) showed that the agglutinating power of colostrum is greater than that of the later milk. This has also been shown repeatedly in the case of women who had suffered from typhoid shortly before parturition (*cp.* Bauer([216]) who gives a full account of the litreature).

5.—*The presence of anti-toxins.*

There is abundant evidence to show that anti-toxins if present in the blood of the mother pass out into the milk. This was first shown by Ehrlich and Wassermann([229]) (1894) who found that if diphtheria anti-toxin were present in the blood of a lactating animal it passed out in the milk in considerable amounts, about one-fifteenth to one-thirtieth that of the blood. If the anti-toxin content of the blood were increased the amount present in the milk also rose.

Most of the work upon the presence of anti-toxins has been carried out in connection with the absorption of these bodies and the production of passive immunity, and will be considered under that heading (*see* p. 70 *et seq.*).

Summarising the results given in Division I. it appears that:—

1. Colostrum contains substances (*a*) acting hœmolytically upon certain blood corpuscles, and (*b*) inhibiting the growth of bacteria, whether through bacteriolysis or agglutination.
2. Later milk may contain traces of these substances but if present at all they are present in very small amounts.

3. The apparent bacteriolytic effect possessed by raw milk seems to be due to its agglutinating powers; this power is lost in the early hours after milking.
4. If specific immune substances are present in the blood of the mother they pass out in the milk but are present in smaller amounts than in the blood.

Division B.—On the Absorption of the so-called "Protective Substances" in Milk.

It has been shown in the preceding division that traces of the bodies concerned in the production of hœmolysis and bacteriolysis may be present in both human and cows' milk, and that agglutinating power is found in fresh milk. Those observers who have worked with colostrum, are all agreed that these powers are greater in colostrum than in the later milk.

If these substances are to be of any value to the infant in producing immunity to disease they must enter the blood stream. The general experience is that these actions are due to some group or body, attached to the proteins of the blood. If therefore these substances are to reach the blood-stream either the protein to which they are attached must be absorbed as such, or the bodies must be able to withstand the action of the digestive juices upon the proteins. In either case the question as to the origin of the protein has to be considered, that is, whether it is native or foreign protein.

The really important question for consideration in the present report is whether the substances are absorbed, the action of the digestive juices being relatively a subsidiary one, since if the substances are not absorbed they cannot be of value to the organism in their specific capacity.

Observers are by no means agreed as to the action of the digestive juices upon these bodies, and this point cannot be looked upon as settled.

Michaelis and Oppenheimer([260]) (1902) found that the protective bodies were destroyed by digestion owing to the breaking down of the proteins to which they were attached. Obermeyer and Pick([279]) (1902) did not however agree with this view, and Sacconaghi([286]) (1904) obtained the production of a precipitin by injecting a solution of protein which had been digested until it no longer gave the protein reactions.

Schlossmann and Moro([292]) (1904) showed that human and cows' protein were quite distinct bodies producing different precipitins on injection, and they concluded that native protein could be absorbed as such from the alimentary canal of the infant, and carry with it the protective properties, whereas foreign protein was broken down in the process of digestion.

Bauer([214]) (1905) reviewing the situation, came to the conclusion that the action of the digestive juices upon these bodies, and the question of the absorption of protein was still undecided. In 1908, however, he stated (at the Meeting of German pediatricians

which took place at Köln, and also private information) that complement if put into an infant's stomach in large amounts, is destroyed, no complement being detected if the fluid is withdrawn five minutes afterwards.

The *absorption* of the protective substances and of protein must now be considered.

The absorption of the bodies concerned in immunity has been studied by many observers, the investigations being for the most part concerned with the production of passive immunity in the suckling animal. Mothers of different species have been injected with anti-toxins both before and after parturition and the blood of the suckling examined for the presence of the anti-toxin, which was injected into the mother. The absorption of protein has been investigated by testing the blood of the young animal by the precipitin reaction.

Absorption of complement and amboceptor have also been sought for.

A few experiments have been carried out on mothers who have been made actively immune to certain bodies, and these will be dealt with separately. As a whole there is considerable evidence to show that in the first days of life protein and its attached properties may be absorbed as such, and thus produce a passive immunity; the length of time during which this capacity lasts after birth depends upon the species of animal.

Later, after the early days of life, absorption of protein from the intestine only occurs in cases of disease or other disturbance of the intestine, or in the presence of excess of protein. This last, however, is probably dependant upon the former factors, since it is a well-known fact that excess of food-material will give rise to intestinal disturbances.

As the greatest amount of work has been carried out in connection with the absorption of *anti-toxins* this will be considered first.

Ehrlich([228]) (1892) showed that temporary immunity to abrin, ricin, robin and tetanus, can be conferred by suckling, upon the young of a mother who is herself immune to these substances. He found that this immunity lasted well, for about a month after birth in the animals (mice) used by him; at the beginning of the third month of life it was much reduced, being absent at the end of that month.

Romer([280]) (1901) injected diphtheria anti-toxin into a pregnant mare shortly before parturition. The anti-toxin content of the blood rose, and after parturition anti-toxin appeared in the milk, in a strength of about one-tenth that of the blood. The foal's blood at time of birth contained no anti-toxin, but after birth it soon appeared and the amount present continued to rise until the 12th day, when it gradually sank. Further injections into the mother gave rise of anti-toxin in the milk, but the anti-toxin content of the foal's blood fell persistently.

Hamburger (1907) immunised female mice to ricin; after parturition one of the immunised mothers suckled the young mice from a normal mother, whilst another immunised mother suckled

her own young. Both families developed a high immunity of about the same degree.

Further, a normal kid was fed by a mother who had been injected with tetanus anti-toxin. The experiment was commenced when the kid was 12 days old, and was continued up to the 24th day; only a small amount of absorption took place. Hamburger concludes that only a part of the protein is absorbed whether of the same species or not.

Much and Römer([267]) (1907) obtained evidence of the absorption of tetanus anti-toxin in the early days of life. They believed that the degree of absorption was greater if the anti-toxin were derived from the blood of the same species than if it were derived from that of a foreign species. A calf was fed from birth with the milk of a mother who had received tetanus anti-toxin: the amount of anti-toxin in the milk was estimated, as was also that in the calf's serum. It was found that about one-twentieth of the anti-toxin present in the milk was absorbed. If, however, the anti-toxin (derived from a horse) were mixed with the milk from a normal mother and fed to the calf, (the same amount of anti-toxin being added as was found to be present in the milk), an absorption of only 1/200 of the amount occurs.

Also if the calf is not allowed to suckle from the immunised mother until the 5th day after birth, only about 1/2000 is absorbed.

Working with Happich, Much([268]) found practically the same for the human infant.

Tetanus anti-toxin was injected into a woman (1) on the day before the confinement, and (2) four days after this event. At birth the infant showed no anti-toxin in the blood, but after four days it had absorbed about one-fourth of the total amount calculated to have been present in the milk, taken from the breast. Between four and eight days of age the infant absorbed one-twelfth of the total anti-toxin present in the milk. If the milk were drawn off and the corresponding amount of anti-toxin (from a horse) added to it, then only about 1/64 was absorbed from the 2-6th days of life, and 1/192 from the 4-8th days.

Much and his collaborators, suggest that the difference is due to a change in the attachment of the anti-toxin, which is taken in by the mother attached to horse protein, and is passed over (as it were) to the human protein in the course of its passage through the human organism, and is thus turned out in the milk as human protein, and is more readily absorbed. Evidence of the presence of horse protein in the milk of the mother could not be obtained by the precipitin reaction, nor by deviation of complement. Further if the human milk containing the anti-toxin was injected into a rabbit, neither human nor horse protein could be found in the milk of that rabbit.

Hamburger([241]) (1907) wrote disagreeing with Much and his fellow-workers. He found that if lactating rabbits are injected with tetanus anti-toxin after parturition this appears in the milk about 26 hours after birth. It appeared as horse anti-toxin, and this could be demonstrated by the ordinary precipitin test; it also

appeared in the blood of the young animal, in about 1/400 the strength of that in the milk, but its presence could only be detected by the usual method of injection of the serum into mice. Fifteen days after parturition the anti-toxin had completely disappeared from the mother's blood, from the milk, and from the blood of the suckling rabbit.

Römer([281]) (1909) carried out further work in this direction, being stimulated thereto by Salge's work upon the absorption of immune body and agglutins (*see* p. 74). He points out that Salge was not using the protein of the same species, and considers this of great importance. He used horse serum and a foal for the purpose of his experiments. The foal was 25 days old when the work was started, and the mother was then injected with tetanus anti-toxin, of known strength and amount. The amount passed out in the milk was estimated on the 4th, 6th, and 8th days after injection. It was calculated that the foal took 15 litres per diem, and working upon this basis it was found to have absorbed one-eighteenth of the total amount of anti-toxin which was present in the milk. Calculating from data obtained upon another horse he found that if the same amount per kilo of body-weight of anti-toxin had been injected into the foal instead of being absorbed from the intestine, the blood would have contained approximately 12 times as much anti-toxin; that is to say the amount of absorption which took place by the intestine was about one-twelfth of what it would have been had the same amount been injected.

The same foal then received milk to which anti-toxin had been added, and no rise of anti-toxin content occurred in the foal's serum, which was now practically free from anti-toxin. He then injected the mother with another dose of anti-toxin and obtained anti-toxin in the milk, but the foal's blood only showed a very slight rise of anti-toxin amounting to 1/10,000 of an anti-toxin unit per cc.

Working with Sames, Römer([282]) (1910) injected a pregnant sheep with tetanus anti-toxin on the 2nd and 6th days before parturition. The blood of the lambs which had suckled from the mother who had been injected, showed anti-toxin up to about 4-6 months of age. Taking his previous results (*cp.* also p. 70) as showing that anti-toxin is not absorbed after the first few days of life these results showed that it is very slowly excreted.

Vaillard([296]) (1896) used mothers which were actively immunised to bacteria, and found that little or no immunity was obtained by suckling. He immunised guinea-pigs to B. Cholera and B. Tetanus, and rabbits to B. Anthracis.

Famulener([231]) (1912) also worked with actively immunised mothers. He immunised pregnant goats to sheep's red corpuscles, and showed that the offspring after suckling had acquired a passive immunity to sheep's corpuscles, the anti-bodies being found in the colostrum. The content of these bodies in the colostrum was often higher than that of the serum of the mother. If the immunisation of the mother was commenced after parturition, no immunity was conferred upon the young animals by

suckling, although anti-bodies could be detected in the milk, but in smaller amounts than in the colostrum. Absorption of the anti-bodies only took place in the early days after birth.

He also found that after the colostral period a high degree of immunity in the mother is required before the anti-bodies pass out in the milk.

These interesting results are in harmony with those already mentioned.

There is a fair amount of evidence dealing with the absorption of protein in the days of life after the early ones. As a whole absorption does not normally appear to take place.

Römer([280]) (1901) found no absorption of anti-toxin from the alimentary canal of adult sheep and guinea-pigs, and believed that in the sheep the anti-toxin was bound by the fœces.

Salge([288]) (1904) fed babies upon human milk to which diphtheria anti-toxin had been added; in a child four days old when the experiment was started no absorption could be detected, even after 20 days: in another child 34 days old and fed for 21 days a negative result was also obtained.

McClintock and King([258]) (1906 and 1909) were able to obtain some degree of passive immunity in children by the absorption of diphtheria anti-toxin from the alimentary canal. In order for this to take place it was found necessary to prepare the intestine and stomach by previous fasting, and by the administration of morphia. Further the anti-toxin had to be given with certain precautions. Unless these precautions were observed there was no absorption of anti-toxin, or so little that it could be considered negligible.

The *absorption of protein* has also been studied, and the protein tested for in the blood by means of the *precipitin* reaction.

Hamburger and Sperk([240]) (1904) failed to obtain absorption of protein in a calf of three days old, or in infants of from 5-13 weeks old, to whom horse serum was given.

Ganghofner and Langer([235]) (1904) found that egg-white was absorbed by young animals during the first few days of life, viz.:—

In puppies it occurred up to the 6th day.
In kittens it occurred up to the 8th day.
In rabbits it occurred up to the 7th day.

In goats absorption was already negative at the 8th day but was found to be positive in one new born kid. In infants they considered that the absorption might last three weeks. If, however, large doses were given absorption could be obtained in older animals. The passage of these substances calls up the appropriate reaction of anti-body formation, which occurs to a fair degree.

Moro([263]) (1906) examined the blood of artificially-fed and diseased infants, by the precipitin reaction. The blood of an atrophic, overfed infant, presumably suffering from some degree of intestinal derangement, gave an intense reaction. He suggests that this may be due to functional derangement of the intestine

due to the feeding with excess of foreign protein. Later he examined 22 more cases of atrophic or otherwise diseased children, and obtained positive results in only two cases. Using the method of complement deviation, in seven cases examined four were positive.

Langer([257]) (1907) showed that colostrum was much richer in antigens than was later milk, and that the blood of young suckling animals in the first days after birth was rich in the same antigens as were found in the milk, these being the same as in the mother's blood. The serum of calves at birth gave no trace of precipitin reaction, but this could be obtained from 6-8 hours after the first feed of colostrum, the reaction increasing up to the second day, and then falling to a lower level. Later the level again rises.

Dealing with adult rabbits Uhlenhuth([295]) (1900) showed that some absorption of egg-white will take place in adult rabbits if the amount given be very large. The reaction was quite definite, but it could not be got to increase.

Mayerhofer and Pribram([259]) (1910) took pieces of the small intestine of quite young guinea-pigs, goats, and rabbits, and also took similar pieces of the intestines from animals of the same age and species, whose intestines had been made abnormal by feeding them upon raw cows' milk. Suitable precautions being taken, the loops of intestine were filled with rennet, tetanus anti-toxin, egg-white, &c., and suspended in saline solution, the amounts of these substances found outside the intestinal mucous membrane being estimated. They found that the permeability of the abnormal intestine was greatly increased.

Van Alstyne and Grant([211]) (1911) made Thierry-Vella fistulae in dogs, and after the fistula was healed the dog was anæsthetised, and egg-white injected into the fistula; the amount of egg-white given is not stated. They obtained evidence of some absorption of the protein because the blood coming away from the loop contained sufficient to produce hypersensitivity in a guinea-pig into which it was injected.

Both Mayerhofer and Pribram's, and Van Alstyne and Grant's experiments are open to the doubt as to the condition of the intestine. It is unlikely that an intestine suspended in saline is in the same condition as an intestine *in situ*, and in the possession of its normal blood-supply. Also in the case of the fistula the condition of the mucous membrane of the fistula is very probably modified, and there is in addition the effect of the anæsthetic.

The evidence upon the *absorption of agglutinins* is not large. Kraus([256]) (1901) found no absorption of typhoid agglutinin in young rabbits whose age is not given, but which appear from the context to have been about three weeks old.

Bertarelli([219]) (1905) induced passive immunity in young animals during the first few days of life, but he did not consider that it was very striking.

Salge([289]) (1907) fed a child nine weeks old, upon the milk of a goat which was immune to typhoid. He obtained no evidence of the absorption of any agglutinin from the intestine of the infant. He concludes that there is no absorption of foreign

protein in the infant after four days of age (*c.p.* his other experiments, p. 73), and he did not feel justified in trying the effect of foreign protein upon a younger infant.

It appears to be well-established that absorption of protein does not take place directly from the alimentary canal after the first few days of life.

Before summarising the whole question, it will be of interest to consider the work which has been carried out in connection with the content of the infants blood at birth as to immune substances, and the gradual development of these substances.

Halban([238]) (1900) took blood from the unbilical cord, and from the mother. He found that the infants blood was much poorer both in agglutinating power and in hœmolytic power, than that of the mother.

Moro([261]) (1901) investigated the complement content of infants blood, in relation to the method of feeding. He found that the bactericidal power of breast-fed children was higher than that of artificially-fed babies, and that this was not merely a question of health because weakly breast-fed babies often had a higher content than healthy artificially-fed ones. Moro states that if the breast-fed baby was put on cows' milk that the bactericidal power fell almost at once. Moro at that time was unable to find any complement in cows' milk, and in human milk. Believing the bactericidal power to be due to direct absorption he was led to the conclusion that complement must be present in the form of a "gen" or alexogenic substance.

Halban and Landsteiner([239]) (1902) showed that the blood of infants at birth is deficient in amboceptor, and likewise in almost all the bodies which produce immunity.

Bertarelli([219]) (1905) tested the power of young animals to form bactericidal and hœmolytic substances by absorption from the intestine. He fed new-born puppies and rabbits on dead cultures of typhoid bacilli and on foreign red corpuscles. There appeared to be no power of forming active immune substances in these young animals during the first four or five days of life. The power then commences, and at the 15-20th day of life these substances may be present in fair amount, not so much inferior to that of the adult. Rather older animals were also fed upon typhoid bacilli, and most of them became very ill, many dying, but agglutinin was formed in the blood. Presumably the bacilli and the red corpuscles gained entrance to the system owing to the derangement of the alimentary canal.

V. Behring([218]) (1903) states that tubercle bacilli can pass the mucous membrane of the young animal in the first few days of life, and later only when large doses are given. This agrees with Bertarelli's work.

Since this date a considerable amount of work has been carried out upon the relationship of the complement content of the blood of infants to their food. The greater part of the work has been carried out by the Munich School of Pediatricians under the guidance of Pfaundler and Moro.

Pfaundler([275]) (speaking on behalf of his school in 1907) said that the blood of 83 infants had been examined, and it had been

found that the blood of the infant at birth contains no amboceptor, but that the complement is nearly as powerful as in the adult. Immediately after birth the complement content falls and its subsequent rise depends somewhat upon the character of the feeding. If breast-fed the content rises, and if artificially-fed it may fall at first, but may rise again afterwards. To some extent the complement varies with the condition of the child, but this is not absolute. Injection of saline may increase the complement content. Later (1908) Pfaundler([277]) and Moro and Potpeschnig([265]) showed that as a whole the healthy infants have a higher complement content than the unhealthy ones, but that in cases of fever, or of the severe condition described by Finkelstein as "Intoxikation" the complement content of the blood is high; should such a condition show a low content the prognosis is very bad. He found that amboceptor is not present in the blood of infants at birth but that the amount rises after birth. In pigs and cows, however, there is hardly any development of amboceptor during the first year. Pfaundler quotes the case of a new-born kid, whose complement content rose during the first few days when it was fed on milk from the breast, but afterwards when fed upon boiled mothers milk the content fell, and the animal very soon developed an attack of constipation and other troubles. On restoring the animal to the breast, the troubles passed away gradually, and at the same time the complement content rose. The weight curve rose steadily the whole time even during the constipation. Bauer pointed out at the time that the results were really due to the sickly condition of the animal, a fall in complement was what occurred in sickly animals, and that this explained the results.

Koch (1909) also found that the complement content of the blood rose in fever; as a whole it tended to increase with improved body-weight and to fall with loss of the same, that is, it depends upon the condition of the cells of the body. In the cases he investigated there was one of a child which was weaned suddenly, and lost complement content; another of a badly nourished artificially-fed child in whom the content improved when put on the breast. He found that the complement content was much less in the blood of the embryo than in the mother, and that amboceptor was not present in the blood of new-born animal, the complement content varying very much.

Kaumheimer([246]) (1909) supplementing the numbers of children worked upon by Moro, came to much the same conclusions as those already given by Pfaundler for his school. As a rule healthy children had high complement content and sickly ones a low one, but there was no absolute rule, and it was impossible to dogmatise upon the matter. Injection of saline raises the hœmolytic power but is is not clear whether this is due to rise of amboceptor or complement.

Gewin([236]) (1909) in Schlossmann's laboratory, investigated the amboceptor-content of new born children for sheep's blood. In breast-fed children this only appears in exceptional cases under six months of age, but in artificially-fed ones it usually occurs earlier. (Amboceptor for sheep's blood is normally present in

human blood.) Gewin thinks that the earlier formation of this amboceptor in the blood of artificially-fed children is due to infection, which induces the formation of amboceptor. The absence of amboceptor in the blood of infants at birth had also been found by Bauer, who stated this fact in the discussion on Pfaundler's paper given at Köln in the previous year. Bauer also pointed out the difficulty of investigating the complement content in infants, owing to the fact that immune sera could not be used. He believed the complement content to depend upon the health of the animal.

Findlay, Foa, and Noegerrath([271]) (1909) estimated the complement content of 98 infants. They found that some did show increase and others did not do so, as their condition improved. One child who was breast-fed and not doing well, with no detectable complement at all, improved at once on artificial feeding. The content increases with the age as does also the amboceptor content. They conclude that the complement content is not a satisfactory basis to use as a foundation for any physiological argument.

Summary of the results of Division B :—

From the evidence given in Division B it appears that—

1. Absorption of protein and hence of the attached immune substances takes place directly during the first few days after birth.
2. Foreign protein is not absorbed as such except in the early days after birth.
3. It is possible that native proteins may be absorbed directly for a longer period.
4. Protein can apparently be absorbed at a later age in significant quantities in cases of disturbance of the alimentary canal, or of presence of excess of protein.

PART IV.—GENERAL SUMMARY AND CONCLUSIONS.

The work described in Part II. shows that most of the ferments, concerning whose presence in milk so much has been heard in the last ten years (*cp.* Escherich) are derived from the bacteria which are found in milk.

There is no evidence to show that uncontaminated milk contains any ferments capable of assisting in the digestion of food by any of the processes of digestion at present known to us.

The only ferments present in uncontaminated milk are those which are well-known to be present in large quantities in the blood, and ferments (whose presence is not firmly established) which act upon substances not known to occur in the processes of digestion.

In milk, the content of ferments which are similar to those which occur in the blood is found to be increased in quantity at periods when the mammary gland is not in a condition of good working activity; such as at the beginning and end of lactation, in mastitis, and in the case of poorly-acting glands.

It is, I believe, universally conceded that milk from a gland in these conditions contains exudate from the blood; the healthier

the gland the less the exudate. It follows, therefore, that the traces of such ferments as are found in the milk under normal conditions, are present because they have passed out from the blood either by filtration or exudation. A precisely similar explanation holds good for the presence in milk of substances concerned in the production of immunity. Here also, it has been shown that these bodies which are normally present in the blood are present in increased amounts in the milk at precisely the same periods as those of its increased ferment content, namely, during the production of colostrum or during mastitis; and that when the mammary gland is in full working order little or none of these bodies appears in the milk. Also if the blood passing to the gland contains specific immune substances these substances appear in small quantities in the milk.

The mammary gland is not only a secretory organ but also an organ admitting of the passage by filtration or exudation, of traces of the substances which are brought to it in the blood-stream.

The value of these materials to the infant evidently depends upon the degree of absorption which takes place from its alimentary canal. From the work quoted in Part II. it appears that absorption of protein and hence of the immune substances which are attached to the protein molecule, may take place directly during the first few days of life. This capacity is however of very short duration, especially for foreign protein, which must be broken down before it can be absorbed.

The oft-repeated assertion of the value to the infant of raw cows' milk fades away when the facts are examined, since, in cows' milk it is found that these so-called "biological substances" are not absorbed in the alimentary canal but are destroyed there.

These considerations also explain the results obtained by those who have investigated the comparative nutritive properties of the raw and boiled milk of a foreign species; these results were fully summarised by me ([255]) in a recent report to the Local Government Board, and it was shown that in dealing with the milk of a foreign species, boiled milk gave perhaps slightly better results than raw milk. In dealing with the milk of a foreign species the real question at issue is that of the chemical changes which take place on heating. The Berlin figures worked up by me, and general clinical experience afford conclusive evidence that raising milk to the temperature of boiling water does not destroy or apparently injure any of the chemical substances necessary for the health of the child. Above this temperature and even at this temperature for any length of time, chemical changes are known to occur. (In this connection the Addendum to this report may be referred to.)

If the milk of the same species be now considered, a somewhat different aspect is put upon the whole question, because there appears to be some degree of evidence that native protein is absorbed as such, for a longer period than foreign protein. The transitory nature of immunity procured by suckling does not however lead to the supposition that this occurs to any extent, and the

evidence all goes to show that the amount of protein which is absorbed as such at any time forms only a small part of the total amount present.

The question finally resolves itself into the chemical value of the food material, and it is this fact which brings about the difference commonly observed between the condition of the average breast-fed baby and the average artificially-fed infant. The substances in the mother's milk are more easily assimilated than those of a foreign species, especially in the early weeks of life, when the organs of the infant are gradually getting into working order. Considering for a moment the almost certain absorption of protein during the first few days of life it would appear to be very important that the organs should receive native protein. Without entering upon the much discussed question of the effect on the infant of the introduction into the blood stream soon after birth of foreign protein, the mere fact that precipitins are formed by the organism upon the introduction of foreign protein for the express purpose of throwing these substances out of action, would of itself seem to indicate that their presence is not desirable. It has been stated that the injection of foreign protein calls forth less resistance in quite young animals than in older ones, but this probably does not mean that such substances are harmless, but that the organs are not yet sufficiently mature to be able to form precipitins. It has been shown by Schlossman and Moro that the proteins of human milk and of human blood are biologically identical, hence the absorption of native protein brings about no disturbance to the young organism, but rather supplies it with ready-made food material, and may thus act as a stimulus to development. That the increased protein content of colostrum may so act is borne out by Birk's([211]) observations. He showed that if an infant is fed upon human colostrum it shows a positive nitrogen balance even although it loses weight during the first few days after birth. If however the normal milk of a wet-nurse is supplied, and not colostrum, the nitrogen balance is negative, even although the infant is receiving the milk of its own species.

The Berlin data obtained for the purpose of the report already referred to showed that the weight-curves of infants fed upon the breast and upon boiled (just raised to 100° C.) milk, increased at approximately the same rate per cent., except during the early weeks of life. It was shown that the marked divergence of the curves at this period could almost certainly be attributed to the method of feeding.

The investigation of the biological properties of milk carried out in this report shows that the weight of evidence suggests the absence of any direct value in the biological substances *per se*. but it also most decidedly shows the paramount importance of providing breast milk for the young animal. It would seem impossible to emphasise this fact too strongly, and all those concerned in the health of infants should aim at obtaining satisfactory breast-feeding for all infants during, at any rate, the early weeks of life.

I am indebted to the Governing Body of the Lister Institute for permission to carry out the original work of the report in the Bio-Chemical Laboratory, and also to Dr. Harden and others for assistance in connection with the work, and take this opportunity of tendering them my thanks.

Addendum.

An account of the work upon the "Biological Properties" of milk would hardly be complete at the present time without a brief reference to the work which has recently been done, and which is still in progress, upon the etiology of beri-beri. This disease, which appears to belong to the "Scurvy" group, is now known to be a disease of deficiency.

The work of Schaumann (Arch. f. Schiffs-und Troppen-Hygiene, May 1912) and of Funk (Journ. of Phys. 1912 and Journ. of State Medicine, June 1912) has shown that the disease can be cured even when the symptoms are well-marked and severe, by the administration of a substance "Vitamine" which has been isolated by Funk from rice-polishings and other materials. This substance is found in a large number of, if not in all, vegetable tissues, and appears to have slightly different properties in the different varieties. It is stated to be thermo-labile, but this property is believed by Funk to be different in the vitamines prepared from different tissues. At present the available data upon this point are somewhat indefinite.

It has been suggested that infantile scurvy may be caused by the deficiency of this substance in milk, it having presumably been destroyed by heat. Funk has prepared this substance from the variety of dried milk known as "Trumilk," so that there is no doubt that it is present in milk. Further, it is almost certain, from our knowledge of beri-beri, that this substance is essential for the well-being of the individual, so that it is presumably necessary for the infant. On this hypothesis the cure of infantile scurvy by the administration of vegetables would be accounted for. It is not unlikely that infantile scurvy may be produced by the deficiency of this substance in milk, either because the milk itself was deficient in the body, or because it had been destroyed by prolonged boiling or by a high temperature.

Before any statement can be made upon the subject a quantitative method for the estimation of the body must be discovered, the length of time of heating and the temperature required for its destruction must be known, and it would also be of value to know the effect upon the substance of keeping, in view of the fact that some of the cases of infantile scurvy appear to have been caused by feeding upon sterilised milk which had been kept.

Further the disease itself should be shown to be curable by the administration of the substance prepared from milk.

Bibliography for Parts I. and II.

(Those papers marked with an asterisk have not been available for personal perusal.)

1. Achard and Clerc. Variations pathologiques du pouvoir amylolytique du sérum sanguin.
   C.R. Soc. de Biol. 1901, vol. 3, p. 708.
2. Alsberg. Beiträge zur Kenntniss der Guajak-Reaktion.
   Arch. f. exp. Pathol. u. Pharm. 1908, vol. 59, suppl., p. 39.

Amoss. (*See* Kastle and Amoss.)

3. Arnold. Nouvelles réactions du lait.
   Journ. de Pharm. 1881, vol. 4, p. 360.

Arnold. *Ibid.* Arch. d. Pharm. 1881, vol. 19, p. 41.

4. Arnold and Menzel. Die Guajakprobe in der Praxis.
   Zeit. f. Fleisch-u. Milch-Hygiene. 1902, p. 205.
5. Arnost. Die Guajak Reaktion der Milch.
   Zeit f. Unters-Nahr. u. Genussm. 1905, vol. 10, p. 538.
6. Austin. The proteolytic enzyme of human milk.
   Journ. of Med. Research. 1908, vol. 19, p. 309.
7. Babcock. The constituents of milk, and some of the conditions which affect the separation of cream.
   Bulletin 18, Agric. Expt. Stat., Wisconsin. 1889.
8. Babcock and Russell. Unorganised ferments of milk.
   Agric. Expt. Stat., Wisconsin. 1897, 14th Report, p. 161.
9. Babcock and Russell. Unorganised ferments of milk.
   Agric. Expt. Stat., Wisconsin. 1898, 15th Report, p. 177.
10. Babcock, Russell and Vivian. Properties of galactase—a digestive ferment of milk.
    Agric. Expt. Stat., Wisconsin. 1898, 15th Report, p. 79.
11. *Ibid.* Distribution of galactase in cows' milk.
    Agric. Expt. Stat., Wisconsin. 1898, 15th Report, p. 87.
12. *Ibid.* Distribution of galactase in different species of Mammalia.
    Agric. Expt. Stat., Wisconsin. 1898, 15th Report, p. 93.
13. *Ibid.* The action of proteolytic ferments on milk, with special reference to Galactase, the cheese-ripening enzyme.
    Agric. Expt. Stat., Wisconsin. 1899, 16th Report, p. 157.
14. Bach and Chodat. Einfluss der Peroxydasen auf die Thätigkeit der Katalase.
    Several papers in the Ber. d. deutschen Chem. Gesell. 1902-1904 and 1906, p. 1670.
15. Bach and Chodat. Untersuchungen über die Rolle der Peroxyde in der Chemie der lebenden Zelle.
    V. Zerlegung der sogenannten Oxydasen in Oxygenasen und Peroxydasen.
    Ber. d.d. chem. Gesell. 1903, vol. 36, p. 606.
16. *Ibid.* Untersuchungen, etc. VI. Uber Katalase.
    Ber. d.d. chem. Gesell. 1903, vol. 36, p. 1756.
17. *Ibid.* Untersuchungen, etc. VIII. Uber die Wirkungsweise der Peroxydase.
    Ber. d.d. chem. Gesell. 1904, vol. 37, pp. 1342-1348; also Bioch. Zentralb. 1903.
18. Bach. Zur Theorie der Oxydasewirkung.
    I. Mangan-und eisen-freie Oxydasen.
    Ber. d.d. chem. Gesell. 1910, vol. 43 (1), p. 364.
19. Bach. Zur Kenntniss der Reduktionsfermente.
    I. Uber das Schardinger-Enzym. (Perhydridase.)
    Bioch. Zeit. 1911, vol. 31, p. 443.
20. Bach. Zur Kenntniss der Reduktionsfermente.
    II. Reduktion der Nitrate durch das System Perhydridase-Aldehyd-Wasser.
    Bioch. Zeit. 1911, vol. 33, p. 282.
21. Barthel. Quoted in Milchztg. 1899, vol. 28, p. 487.
22. Barthel. Stérilisation du lait au moyen de peroxyde d'Hydrogène.
    Rév. gén. du lait. 1903, vol. 2, p. 285.

23. Barthel. La destruction de la peroxydase du lait par H_2O_2 a 50° C.
Rev. gen. du lait. 1907, vol. 6, p. 534.
24. Barthel. Reduktaseprobe bei Milch.
Zeit. Unters-Nahr. u. Genussm. 1908, vol. 15, p. 385.
25. Barthel. Die Reduktaseprobe.
Milchztg. 1910, vol. 39, p. 25.
26. Barthel. Die Reduktaseprobe.
Zeit. f. Unters-Nahr. u. Genussm. 1911, vol. 21, p. 513.
27. Becamp. Sur la cause du dégagement de l'oxygène de l'eau oxygénée par la fibrin; influence de l'acide cyanhydrique tarissant l'activité de la fibrin.
Contes Rendus. 1882, vol. 95, p. 925.
28. Becamp. Sur les microzymases comme cause de la décomposition de l'eau oxygénée par les tissues des animaux et des végétaux.
Contes Rendus. 1882, vol. 94, p. 1653.
29. Becamp. Action de l'eau oxygénée sur la matière colorante du sang et sur l'hematosine.
Contes Rendus. 1882, vol. 94, p. 1720.
30. Becamp. Sur la zymase du lait de femme.
Contes Rendus. 1883, vol. 96, p. 1508.
31. Bellei. Intorno ad una speziale reazione del latte.
Giornace della Societa Italiana di Igiene. 1904, vol. 26, p. 52.
Ref. Zentralb. f. Bakt. II. 1904, vol. 12, p. 548.
32. Bernheim-Karrer. Untersuchungen. über das Fibrinferment der Milch.
Zentralb. f. Bakt. 1902, vol. 31, p. 388.
33. Bert and Regnard. Action de l'eau oxygénée sur les matières organiques et les fermentations.
Contes Rendus. 1882, vol. 94, p. 1383.
34. Bertrand. Sur l'intérvention du manganèse dans les oxydations provoquées par la laccase.
Contes Rendus. 1897, vol. 124, p. 1032.
35. Bertrand. Sur l'action oxydant des sels manganeux et sur la constitution chemique des oxydases.
C.R. 1897, vol. 124, p. 1355.
36. Bial. Uber die diastatische Wirkung des Blut-u. Lymph-Serums.
Pflüger's Arch. 1892, vol. 52, p. 137.
37. Bier. Uber die Katalase in der Milch.
Maly's Tierch. 1905, vol. 35, p. 230.
38. Biolchini and Luzzati. Uber die Answesenheit einiger löslichen Fermente in der Milch. Given at the 4th Congress of Ital. Ped. in Florence. 1901, Ref. Arch. f. Kinderh. 1902, vol. 33, p. 460.
39. Boekhout and De Vries. Uber die Reifungsprozess der Edamer Käse.
Zentralb. f. Bakt. II. 1899, vol. 5, p. 304.
40. *Ibid.* 1901, vol. 7, p. 817.
41. Bordas and Touplain. Sur les diatases du lait.
Contes Rendus. 1909, vol. 148, p. 1057.
42. Bordas and Touplain. Contribution a l'étude des réactions dues a l'état colloidal du lait cru.
Contes Rendus. 1910, vol. 150, p. 341.
43. Bourquelot. Remarques sur les matières que l'on peut rencontrer chez les êtres vivants.
Contes Rendus. 1897, vol. 123, p. 315; and C.R. Soc. de Biol. 1897, vol. 4, pp. 403, 687.
44. Brand. Uber die praktische Bedeutung der Reduktionsfähigkeit der Milch.
Münch. Med. Wochens. 1907, vol. 54. I. p. 821.
45. Bredig and Sommer. Anorganische Fermente und die Schardingersche-Reaktion.
Zeit. f. Physik. Chemie. 1910, vol. 70, p. 34.
46. Brüning. Uber das Verhalten des Schwefels zur Milch.
Zeit. f. exp. Pathol. u. Ther. 1906, vol. 3, p. 157.
47. Burri and Kursteiner. Zur Klärung der Anschauungen über die reduzierenden Eigenschaften der Kuhmilch.
Milchw. Zentralb. 1912, vol. 8, pp. 40, 68, 101, 134, 168. (5 papers.)

48. Burri and Staub. Ein neuer Apparat zur Bestimmung der Milch katalase.
Zeit. f. Unters. Nahr. u. Genussm. 1909, vol. 17, p. 88.

49. Butterberg. Zur Untersuchung der pasteurisierten Milch.
Zeit. f. Unters. Nahr. u. Genussm. 1906, vol. 11, p. 377.

50. Camus. Action du lait sur la coagulation du sang.
Journ. de Phys. et Path. gen. 1901, vol. 3, p. 27.

51. Cathcart and Hahn. Uber die reduzierenden Wirkungen der Bakterie.
Arch. f. Hyg. 1902, vol. 44, p. 295.

52. Chick. Sterilisierung von Milch durch Wasserstoffsuperoxyd.
Zentralb. f. Bakt. II. 1901, vol. 7, p. 705.

53. Chlopin. Ein neues Reagens auf Ozon.
Zeit. f. Unters. Nahr. u. Genussm. 1902, vol. 5, p. 504.

Chodat. (*See* Bach.)

Clerc. (*See* Achard.)

54. Desmoulières. Sur le ferment du salol contenu dans certains laits.
Journ. de pharm. chim. 1903, vol. 17, p. 232; and Contes Rendus. 1903, vol. 137, p. 337.

55. Dubourg. Recherches sur l'amylase de l'urine.
Annales de L'inst. Pasteur. 1889, vol. 3, 304.

56. Dupouy. Moyen de différencier le lait cru du lait bouilli.
Journ. de pharm. 1903, vol. 17, p. 483.

*57. Duclaux. Traité de Microbiologie. Vol. II. Diastases, toxines, et venins.
Paris. 1899, p. 392.

58. van Eck. Uber das Erhalten der Kuhmilchperoxydase beim Erhitzen.
Zeit. f. Unters. Nahr.-u. Genussm. 1911, vol. 22, p. 393.

59. Ehrlich. Uber Immunität durch Vererbung und Säugung.
Zeit. f. Hyg. 1892, vol. 12, p. 183.

60. Engler and Wöhler. Pseudocatalytische Sauerstoffübertragung.
Zeit. f. anorg. Chem. 1902, vol. 27, p. 1.

61. Escherich. Zur Kenntniss der Unterschiede zwischen der natürlichen und künstlichen Ernährung des Säuglings.
Wiener Klin Wochens. 1900, vol. 13, p. 1184.

62. Faitelowitz. Zur Kenntniss der Katalase in Milch, und deren Bedeutung für die Milchkontrolle.
Milchw. Zentralb. 1910, vol. 6, pp. 299, 361 and 420.

63. Freudenreich. Uber das in der Milch verhandene unorganisierte Ferment, die sogenannte Galactase.
Zentralb. f. Bakt. II. 1900, vol. 6, p. 332; and Milchztg. 1900, vol. 29, p. 245.

64. Friedjung and Hecht. Uber Katalase und Fermentwirkung der Milch.
Arch. f. Kinderh. 1903, vol. 37, p. 346.

65. Fraenckel and Hamburger. Uber Diastase. I. Versuche zur Herstellung von Rein—diastase und deren Eigenschaften.
Beitr. d. chem. Physiol. u. Pathol. 1906, vol. 8, p. 389.

66. Gerber and Ottiker. Zur Katalase Bestimmung der Milch.
Milchw. Zentralb. 1910, vol. 6, p. 316.

Gerum. (*See* Paal and Gerum.)

67. Giffhorn. Untersuchungen über Enzyme in der Kuhmilch
Inaug. Dissert. Hannover, 1909. Ref. Rév. gén. du lait. 1911, vol. 8, No. 22.

68. Gillet. Le ferment oxydant du lait.
Journ. de Phys. et de Path. gen. 1902, p. 439.

Gillet. (*See* Marfan and Gillet.)

69. Glage. Die Guajakprobe in der Praxis.
Zeit. f. Fleisch-u. Milch-Hygiene. 1901, p. 162.

70. Grimmer. Beiträge zur Kenntniss der Enzyme der Milchdruse.
Milchw. Zentralb. 1910, vol. 6, p. 142.

71. Grimmer. Zur Kenntniss der Milchperoxydase
Milchw. Zentralb. 1911, vol. 7, p. 295.

72. Grimmer. Liegt den Erscheinungen der sog. Peroxydase ein Ferment zugrunde?.
Zeit. f. Unters. Nahr-u. Genussm. 1911, vol. 15, p. 676.
73. Grimmer. Bemerkungen zu der Arbeit von W. D. Kooper, "Untersuchungen uber die Katalase," etc.
Milchw. Zentralb. 1911, vol. 17, pp. 314 and 1712.
74. Van der Haar. Untersuchungen über Pflanzen-peroxydasen. I. Eine neue method der Peroxydase-Gewinnung. II. Die Hedera Peroxydase. Ein Gluco-proteide.
Ber. d. d. chem. Gesell. 1910 (2), vol. 43, pp. 1321 and 1327.
Hahn. (*See* Cathcart and Hahn.)
Hamburger. (*See* Fraenckel and Hamburger and Moro and Hamburger.)
75. Harden and Lane-Claypon. Occurrence of ferments in the sterile milk collected by milking-tube from cows and goats.
Journ of Hyg. 1912, vol. 12, No. 2, p. 143.
76. Hecht. Die Reduktion als Lebensfunktion der Milch.
Arch. f. Kinderh. 1904, vol. 38, p. 349.
Hecht. (*See* Friedjung and Hecht.)
Hertel. (*See* Tjaden, Koske and Hertel.)
77. Hesse. Weitere Versuche betreffend Nachweis von gekochter Milch.
Milchw. Zentralb. 1909, vol. 5, p. 544.
Hesse. (*See* Kooper.)
78. Heygendorff and Meurer. Zur Katalasebestimmung.
Milchw. Zentralb. 1910, vol. 6, p. 529.
79. Hippius. Biologisches zur Pasteurisierung.
Jahrb. f. Kinderh. 1905, vol. 61, p. 365.
80. Hoeffter. Uber die Wirkung des Schwefels auf Eiweisskorper. II. Das Verhalten der Sekrete gegen Schwefel.
Beitr. d. chem. Physiol. u. Path. 1904, vol. 5, p. 213.
81. Ibrahim. Neuere Forschungen über die Verdauungs-Physiologie des Säuglingsalters.
Verd. d. Gesell. f. Kinderh. Köln. 1908, p. 21.
82. v. Itallie. Sur les catalases du sang.
C.R. Soc. de Biol. 1906, vol. 60, p. 148.
83. Jensen. De l'origine des catalases et réductases du lait de vache.
Rév. gén. du lait. 1906, vol. 6, pp. 33, 56 and 85.
84. Jolles. Beiträge zur Kenntniss der Frauenmilch.
Zeit. f. Biol. 1904, vol. 45, p. 248.
*85. Kastle and Amoss. Variations in the Peroxidase activity of the Blood in Health and Disease.
Bulletin 31, Hygienic Lab., Washington.
86. Kastle and Porch. The peroxidase reaction of milk.
Journ. of biol. chem. 1908, vol. 4, p. 301.
87. Klebs. Die pyrogene Substanz.
Zentralb. f. med. Wissen. 1868, p. 417.
88. Koning. Biologische und Biochemische Studien uber Milch. V. Die Enzyme.
Milchw. Zentralb. 1906, vol. 2, pp. 517, 531; and 1907, vol. 3, pp. 41, 53.
89. Koning. *Ibid.* Das Pasteurisieren.
Milchw. Zentralb. 1911, vol. 7, p. 97.
*90. Koning. Die Storch'sche Reaktion.
Verl. Mitt. Pharm. Weekblad. 1905 and 1906, vol. 43, p. 741.
91. Kooper. Beitrag zur Kenntniss der Oxydasen und Reduktasen der Kuhmilch.
Zeit. f. Unters Nahr-u. Genussm. 1910, vol. 20, p. 565.
92. Kooper and Hesse. Methoden des Nachweises einer stattgehabter Erhitzung von Milch und Molkereiprodukten, speziell in der Butter.
Milchw. Zentralb. 1910, vol. 10, pp. 412 and 468.
93. Kooper and Hesse. Liegt den Escheinungen der sog. Peroxydase ein Ferment zugrunde?.
Zeit. f. Unters Nahr-u. Genussm. 1911, vol. 21, p. 385.
94. Kooper. Untersuchungen über die Katalase.
Milchw. Zentralb. 1911, vol. 7, p. 264.

95. Kooper. Erwiederung auf die Bemerkungen von Dr. Grimmer, etc.
Milchw. Zentralb. 1911, vol. 7, p. 411.
Koske. (*See* Tjaden Koske and Hertel.)
96. Kowalewsky. Uber das Verhalten der Milch zum Guajakharz.
Zentralb. d. med. Wissen. 1890, pp. 145 and 162.
Rev. de Sc. Medicales. 1891, vol. 38, p. 45.
Kursteiner. (*See* Burri and Kursteiner.)
97. Lam. Die Prüfung der Marktmilch.
Maly's Tierch. 1906, vol. 36, p. 232.
*98. Landtsheer and Vandevelde.
Arch. de méd. des Enfants. 1903, July.
99. Langstein. Das Eisen bei der natürlichen und künstlichen Ernährung.
Jahrb. f. Kinderh. 1911, vol. 74, p. 536.
Lane-Claypon. (*See* Harden and Lane-Claypon.)
100. Leffmann. Note on the test for distinguishing boiled from unboiled milk.
Analyst, 1898, p. 85.
*101. Leperre. Quoted by Vandevelde.
8th VI. Nat. on Geneesk. Congres., Antwerpen, 1904.
*102. Lepine. Entstehung und Verbreitung des tierischen Zuckerfermentes.
Ber. d.d. Sachs. Gesell. d. Wissen. 1870.
103. Lepinois. Sur l'existence dans l'organisme de plusieurs matières albuminoides décomposant l'eau oxygénée.
C.R. Soc. de Biol. Vol. 1, 1899, p. 429; and Thèse de Doctorat. Paris, 1899.
104. Loew. Eine Bemerkung uber Katalase.
Zeit. f. Biol. 1902, vol. 43.
105. Loew. Catalase. A new enzyme of general occurrence, with special reference to the tobacco plant.
U.S. Dept. of Agriculture. Report 68, Washington. 1901.
106. Loew. Spielt Wasserstoffsuperoxyd eine Rolle in der lebenden Zelle?
Ber. d.d. chem. Gesell. 1901, vol. 35, p. 2487.
Luzzati. (*See* Biolchini.)
107. Marfan. Allaitement natural et allaitement artificiel; hypothèses sur le rôle des zymases du lait.
La Presse Médicale. 1901, p. 13.
108. Marfan. L'Amylase du lait.
Bull. de la Soc. de Péd. de Paris. 1902, p. 113.
109. Marfan and Gillet. Uber zwei Fermente der Milch.
Monats f. Kinderh. 1902, vol. 1, p. 57.
Menzel. (*See* Arnold and Menzel.)
Merklen. (*See* Nobécourt and Merklen.)
Meurer. (*See* Heygendorff and Meurer.)
110. J. Meyer. Bermerkungen über die Fermente der Milch.
Arb. a.d. Kaiserl. Gesundheitsamt. 1910, vol. 34, p. 115.
111. Miele and Willem. A propos d'une diastase lactique dédoublement le salol.
Contes Rendus. 1903, vol. 137, p. 135.
112. Monvoisin. Sur quelques diastases du lait.
Rév. gén. du lait. 1907, vo. 6, p. 265.
113. Moro. Die Fermente der Milch.
Jahrb. d. Kinderh. 1902, vol. 56, p. 361.
114. Moro. Untersuchungen über diastatisches Enzym in den Stühlen von Säuglingen und in der Muttermilch.
Jahrb. d. Kinderh. 1898, vol. 47, p. 342.
115. Moro. Zur Charakteristikch des diastatischen Enzymes in der Frauenmilch.
Jahrb. d. Kinderh. 1900, vol. 52, p. 524.
116. Moro and Hamburger. Ein Beitrag zur Kenntniss der Unterschiede zwischen Menschenmilch und Kuhmilch.
Wiener Klin. Wochens. 1902, vol. 15, p. 121.

117. Moore and Whitley. The Properties and Classification of the oxidising enzymes, etc.
Biochemical Journ. 1909, vol. 4, p. 136.
118. Müller. Die Reduktionsprobe. Ein mittel zur Beurteilung des Frischezustandes der Milch.
Arch. f. Hyg. 1906, vol. 56, p. 108.
119. Neisser and Wechsberg. Uber eine neue einfache Methode zur Beobachtung von Schädigungen lebenden Zellen.
Münch. Med. Wochens. 1900, vol. 47, p. 1261.
120. Neumann-Wender. Die Enzyme der Milch.
Oesterr. Chem. Ztg. 1903, vol. 6, p. 1.
121. Neumann-Wender. Der Mechanismus der Guajak-Reaktion.
Oesterr. Chem. Ztg. 1904, vol. 7, p. 532.
122. Neumann-Wender. Die Oxydasen. Chemiker-zeitung. Vol. 26, pp. 1217 and 1221.
123. Nobécourt and Merklen. Un ferment du lait de femme et du lait d'Anesse.
Révue mens. des mal. de l'enfance. 1901.
124. Nobécourt and Sévin. Le ferment amylolytique chez les nourrices et les vaches laitières.
Bull. de la Soc. de Péd. de Paris. 1902, vol. 4, p. 20.
125. Nobécourt and Sévin. Le ferment amylolytique che du sang chez les enfants normaux.
C.R. Soc. de Biol. 1901, vol. 3, p. 1068.
126. Nobécourt and Sévin. Le ferment amylolytique du serum sanguin chez l'enfant normal et chez l'enfant malade.
Rév. mens. des mal. de l'enfance. 1902, vol. 20, p. 25.
127. Nordmann. Uber ein positiven chemischen Befund bei Unverträglichkeit der Muttermilch.
Monats. f. Geburts. u. Gyn. 1902, vol. 15, p. 152.
*128. Oppenheimer. Weiteres zur Schardinger'schen Reduktionsprobe.
Arb. a.d. Inst. exp. Therap., Frankfurt. 1908, vol. 4, p. 75.
Ottiker. (*See* Gerber and Ottiker.)
129. Paal and Gerum. Ber. d.d. chem. Gesell. 1908, vol. 41, p. 805.
*130. Payen and Persoz. Memoire sur la diastase, les principaux produits de ses reactions, etc.
Ann. de Chim. et Phys. 1833, vol. 35, p. 73.
Persoz. (*See* Payen and Persoz.)
131. Plimmer, Aders. On the presence of lactase in the intestines of animals, etc.
Journ. of Phys. 1906, vol. 35, p. 20.
Porch. (*See* Kastle and Porch.)
132. Pozzi-Escot. Dédoublement disastasique du salol.
Contes Rendus. 1903, vol. 136. May 11th.
133. Raudnitz. Uber die sogenannte Ferment-Reaktion der Milch.
Centralb. f. Phys. 1898, vol. 12, p. 790.
134. Raudnitz. Beiträge zur Kenntniss der oxydativen Fermente und der Superoxydasen.
Zeit. f. Biol. 1901, vol. 42, p. 91.
135. Raudnitz. Sammelreferate.
Monats. f. Kinderh. 1902, vol. 1, p. 338.
136. Raudnitz. Ergebnisse der Physiologie. 1903, vol. 2, p. 274. Abt. 11 Fermente.
Regnard. (*See* Bert and Regnard.)
137. Reinhard and Seibold. Das Verhalten der Schardingerschen Reaktion gegenuber Colostral-Milch und Rahm.
Bioch. Zeit. 1911, vol. 31, p. 295.
138. Reinhard and Seibold. Das Schardingerschen Enzym in Milch von euterkranken Kuhen.
Bioch. Zeit. 1911, vol. 31, p. 385.
139. Reiss. Die Katalase der Milch.
Zeit. f. Klin. Medizin. 1905, vol. 56, p. 1.
*140. Renard. La conservation du lait par l'eau oxygénée.
Bioch. Zentralb. 1903, vol. 2, p. 544.

141. Römer and Sames. Beiträge zur Schardingerschen Reaktion der Kuhmilch.
Zeit. Unters. Nahr.-u. Genussm. 1910, vol. 20, p. 1.
142. Römer. Zur Schardinger-Reaktion der Kuhmilch.
Bioch. Zeit. 1912, vol. 40, p. 5.
*143. Rösing. Untersuchungen über die Oxydation von Eiweiss in Gegenwart von Schwefel.
Inaug. Dissert. Rostock. 1891.
144. Rothenfüsser. Uber den Nachweis von Fermenten unter besondere Berücksichtigungen der Milch.
Zeit. Unters. Nahr-u. Genussm. 1908, vol. 16, p. 63.
145. Rothenfüsser. Methoden des Nachweises einer stattgehabter Erhitzung der Milch, etc.
Milchw. Zentralb. 1910, vol. 6, p. 468.
146. Rullmann. Uber Pasteurisieren und Sterilisieren der Milch im allgemein, etc.
Zeit. Unters. Nahr-u. Genussm. 1903, vol. 6, p. 603.
147. Rullmann. Uber Reaktionen des Oxydierenden Enzymes der Kuh-u Frauenmilch.
Zeit. f. Unters. Nahr-u. Genussm. 1904, vol. 7, p. 81.
148. Rullmann. Die Schardingerscher-Reaktion der Milch.
Bioch. Zeit. 1911, vol. 32, p. 446.
149. Rullmann. Uber den Enzym-und Streptokokken-Gehalt aseptisch-gewonnener Milch.
Arch. f. Hyg. 1911, vol. 73, p. 81.
Russell. (*See* Babcock and Russell.)
150. Sames. Uber einige Farbenreaktionen zur Unterscheidung der erhitzter von der rohen Kuhmilch.
Milchw. Zentralb. 1910, vol. 6, p. 462.
Sames. (*See* Römer and Sames.)
151. Sassenhagen. Uber die biologische Eigenschaften der Colostral-und Mastitis Milch.
Arch. f. Kinderh. 1910, vol. 53, p. 281.
152. Sarthou. Du rôle que parait jouer le fer dans la schinoxydase.
Journ. de Pharm. 1900, vol. 2, p. 583.
153. Sarthou. Sur la presence d'une anaeroxydase et d'une catalase dans le lait de vache.
Journ. de Pharm. Vol. 30, 1909, p. 350; and Contes Rendus. 1909, vol. 149, p. 809; and *Ibid.* 1910, vol. 150, p. 119.
154. Sarthou. Rôle du fer dans les phénomènes d'oxydation. Oxydases artificielles à base de fer.
Journ. de Pharm. 1911, vol. 3, p. 49.
155. Sartory. 1. Sur les proprietes oxydasiques d'une eau minérale.
C.R. Soc. de Biol. 1911, vol. 70, p. 522.
2. Action de quelques sels sur la teinture de guaiac.
C.R. Soc. de Biol. 1911, vol. 70, p. 700.
3. Sur quelques réactions fournis par la teinture de guaiac.
C.R. Soc. de Biol. 1911, vol. 70, p. 895.
156. Schaer. Uber verschiedene Reaktionen der Kupferoxydase bei Gegenwart von Cyanverbindungen.
Ber. d.d. chem. Gesell. 1869, vol. 2, p. 730.
157. Schardinger. Uber das Verhalten der Kuhmilch gegen Methylenblau und seine Verwendung zur Unterscheidung von ungekochter und gekochter Milch.
Zeit. f. Unters. Nahr-u. Genussm. 1902, vol. 5, p. 1113.
158. Schardinger. Einige Bemerkungen zu den mir im Laufe des Jahres 1903 bekannt geworden Veröffentlichungen, etc.
Chemztg. 1904, vol. 28, p. 704; and Zeit. f. Unters. Nahr. u. Genussm. 1905, p. 556.
159. Schern. Uber die Schardingersche Reaktion der Milch.
Bioch. Zeit. 1909, vol. 18, p. 261.
160. Schlossmann. Verh. d. Gesell. Kinderh. Hamburg. 1902.
161. Schroeter. Vergleichende Prüfung bacteriologischer und biochemischer Methode zur Beurteilung der Milch.
Zentralb. f. Bakt. II. 1911, vol. 32, p. 181.

162. Schweitzer. Milchhygienische Studien.
Centralb. f. Bakt. II. 1903, vol. 10, pp. 501 and 563.
Seibold. (*See* Reinhard and Seibold.)
163. Seibold. Uber den Keimgehalt unter aseptischen Kautelen gewonner Milch, und dessen Bedeutung für die Praxis.
Centralb. f. Bakt. I. 1910, vol. 55, p. 301.
164. Seligmann. Uber den Einfluss einiger Aldehyde, besonders des Formalins auf die Oxydationsfermente der Milch, etc.
Zeit. f. Hyg. 1905, vol. 50, p. 97.
165. Seligmann. Über die Reduktase der Kuhmilch.
Zeit. f. Hyg. 1905, vol. 50, p. 97.
166. And *Ibid.* Zeit. f. Hyg. 1906, vol. 52, p. 161.
167. And *Ibid.* Zeit f. Hyg. 1908, vol. 58, p. 1.
168. Seligmann. Uber den Nachweis stattgehabter Erhitzung der Milch.
Zeit. f. angew. Chemie. 1906, vol. 19, p. 1540.
169. Senter. Das Wasserstoffsuperoxyd-zersetzende Enzym des Blutes.
Zeit. f. Phyik. Chemie. 1903, vol. 44, p. 257.
Sévin. (*See* Nobécourt and Sévin.)
170. Siegfeld. Uber den Nachweis einer Erhitzung der Milch.
Milchztg. 1901, p. 772.
171. Siegfeld. Nachweis einer Erhitzung der Milch.
Zeit. f. angew. Chemie. 1903, vol. 16, p. 764.
172. Smidt. Uber die Fähigkeit der Milch, Methylenblau zu reduzieren.
Hyg. Rund. 1904, vol. 14, p. 1137.
173. Smidt. Über die sogenannte Reduktase der Milch.
Arch. f. Hyg. Vol. 58, 1908, p. 313.
174. Snyder. The digestive action of milk.
12th Annual Report Agric. Expt. Stat., Minnesota. 1904.
Sommer. (*See* Bredig and Sommer.)
175. K. Sommerfeld. Uber Säuglingsmilch mit besonderer Berücksichtigung des Much-und Römerschen Verfahrens.
Giessener Vet. med. Diss. 1907.
Ref. Sammelreferate Monats. f. Kinderh. 1908, vol. 7, p. 385.
176. P. Sommerfeld. Die Frage der reduzierenden Eigenschaften der Milch.
Hyg. Centralb. 1908, vol. 4, p. 1.
177. Spindler. Beiträge zur Kenntniss der Milchkatalase.
Bioch. Zeit. Vol. 30, 1911, p. 384.
178. Spolverini. Présence des ferments soluble dans le lait.
Arch. de méd. des enfants. 1901, vol. 7, p. 129.
179. Spolverini. Sui fermenti del latte, etc.
Il Policlinico. Vol. 8, 1902, p. 707.
*180. Spolverini. Rev. d'hyg. et de méd. inf. 1902, vol. 1, Nos. 3 and 4.
181. Spolverini. Le ferment oxydant du lait.
Rev. d'hyg. et de méd inf. 1904, vol. 2, No. 2.
Staub. (*See* Burri and Staub.)
182. Stoklasa. Uber der Isolierung gärungs-erregender Enzyme aus Kuh-u. Frauenmilch.
Arch. f. Hyg. 1907, vol. 50, p. 165.
183. Storch. Eine Methode sur Unterscheidung von pasteurisierten und nicht-pasteurisierten Milch.
(1.) Ber. des Versuchst. d. K. Vet. u. Landb. hochs. Kopenhagen.
(2) Milchztg. 1898, vol. 27, p. 374.
Strich. (*See* Wohlgemuth and Strich.)
Sugg. (*See* Vandevelde, de Waele, and Sugg.)
184. Thiemich. Uber die Stor'sche Reaktion der Frauenmilch.
Monats. f. Geb. u. Gyn. 1903, vol. 16, p. 32.
185. Tjaden, Koske, and Hertel. Zur Frage der Erhitzung der Milch, mit besonderen Berücksicktigung der Molkerein.
Arb. a.d. Kaiserl. Gesundheits. 1901, vol. 18, p. 219.
186. A. Torday. Der Einfluss physikalischer und chemischer Faktoren auf die Katalyse der Frauenmilch.
Maly's Tierch. 1907, vol. 37, p. 264.
187. A. and E. Torday. Uber die Katalase der Frauenmilch.
Jahrb. d. Kinderh. 1908, vol. 67, p. 277.
Touplain. (*See* Bordas and Touplain.)

188. Trommsdorff. Zur Frage der reduzierenden Eigenschaften der Milch und der Schardinger'schen Reaktion.
Zentralb. f. Bakt. 1909, I., vol. 49, p. 291.

189. Utz. Der Nachweis einer Erhitzung der Milch.
Zeit. f. angew. Chemie. 1903, vol. 16, p. 871

190. Utz. Weitere Beiträge zum Nachweis von gekochter und ungekochter Milch.
Milchztg. 1903, vol. 32, p. 417.

191. Utz. Zur Untersuchung von roher und gekochter Milch.
Milchztg. 1903, vol. 32, p. 594; also Milchztg. 1902, p. 145; and 1903, p. 129; and Oesterr. Chem. Ztg. 1904, p. 916.

192. Utz. Entsteht beim Kochen der Milch Schwefelwasserstoff?
Milchztg. 1903, vol. 32, p. 354.

193. Utz. Uber Reaktionen des oxydierenden Enzyms der Kuh-u. Frauenmilch.
Oesterr. Chem. Zeit. 1904, vol. 7, pp. 389 and 533; and Zeit. f. Unters Nahr. u. Genussm. 1905, vol. 9, p. 556.

194. A. J. J. Vandevelde, de Waele and Sugg. Uber proteolytische Enzyme der Milch.
Hofmeister's Beiträge. 1904, vol. 5, p. 571.

195. A. J. J. Vandevelde. Untersuchungen über die Proteolyse der Milch.
Bull. Soc. chim. de Belg. 21, p. 434, ref. Maly, vol. 37, 1907, p. 295.

196. A. J. J. Vandevelde. Nouvelles recherches sur les ferments solubles du lait.
Memoires couronnées de l'acad. sc. Belg. 1907.

197. A. J. J. Vandevelde. Uber Milchzucher zerstörende Enzyme in der Milch.
Bioch. Zeit. 1908, vol. 11, p. 61.

198. Vaudin. Beurteilung der Frische der Milch durch Indigolösung.
Rep. de Pharm. 1897, p. 538, ref. Maly, 1897, p. 232.

199. R. v.d. Velden. Die " Katalase " der Frauenmilch.
Bioch. Zeit. 1907, vol. 3, p. 403.

Vivian. (*See* Babcock, Russell and Vivian.)

de Vries. (*See* Boekhout and de Vries.)

200. Waentig. Literatur Ubersicht betr. die Veranderungen der Kuhmilch beim Erhitzen.
Arb. a.d. Kaiserl. Gesundh. 1907, vol. 26, p. 582.

201. Waentig. Die Peroxydase Reaktion der Kuhmilch, etc.
Arb. a.d. Kaiserl. Gesundh. 1907, vol. 26, p. 464.

202. Warfield. A peptide-splitting enzyme in woman's milk.
Journ. of Med. Research. 1911, vol. 25, p. 235.

203. Weber. Arnold's Guajakprobe zur Unterscheidung roher und gekochter Milch.
Milchztg. 1902, pp. 657 and 673.

204. Weber. Untersuchungen über die Kolostralmilch der Kuh, der Ziege, und des Schafes.
Milchw. Centralb. 1910, vol. 6, pp. 433, 481, 543.

Wechsberg. (*See* Neisser and Wechsberg.)

Whitley. (*See* Moore and Whitley.)

Willem. (*See* Miele and Willem.)

Wöhler. (*See* Engler and Wohler.)

205. Wohlgemuth and Strich. Untersuchungen über die Fermente der Milch, und über deren Herkunft.
Sitzungsber d.k. preus. Akad. der Wissen. Berlin. 1910, vol. 1, p. 520.

206. Wirthle. Ein neues Verfahren zum Nachweis von gekochter und ungekochter Milch.
Chem. Ztg. 1903, p. 432.

207. Wynter Blyth. The detection and estimation of preservatives in milk.
Analyst. 1901, vol. 25, p. 128.

208. Zaitschek. Vergleichende Untersuchungen über den Gehalt an Eiweiss und Stärke-losenden Enzymen, verschiedenen Milcharten.
Pflüger's Arch. 1904, vol. 104 (4), p. 539.

209. Zink. Milchztg. 1903.

210. Zweifel. Untersuchungen über den Verdauungs - apparat der Neugeborenen.
Centralb. f. med. Wissen. 1874, vol. 12, p. 939.

Bibliography for Parts III. and IV.

211. Van Alstyne and Grant. Absorption of albumin without digestion.
Journ. of Med. Research. 1911, vol. 25, p. 399.

212. Bartelli. Beitrag zum Studium Bakteriozidine der Milch.
Michw. Zentralb. 1909, vol. 5, p. 462.

213. Basenau.
Arch. f. Hyg. 1895, vol. 23, p. 44.

Basenau.
Arch. f. Hyg. 1895, vol. 23, p. 177.

214. Bauer. Uber die Durchgängigkeit des Magendarmkanals für Eiweiss und Immunkörper, etc.
Arch. f. Kinderh. 1905, vol. 42, p. 399.

215. Bauer. Uber biologische Milch-Differenzierung.
Münch. Med. Wochens. 1908, vol. 55, no. 16, p. 847.

216. Bauer. Die Biologie der Milch.
Ergebn. f. Journ. Med. Vol. 5, p. 183.

217. Bauer and Sassenhagen. Ein neues Verfahren zum Nachweiss der Mastitis-Milch.
Med. Klinik. 1909, p. 1927.

218. v. Behring. Uber Lungenschwindsucht-Entstehung und Tuberkulose-Bekämpfung.
Deut. Med. Wochens. 1903, vol. 29, p. 617.

219. Bertarelli. Uber aktive und passive Immunisation der Neugeborenen und Säuglinge auf dem Wege der Verdauungsorgane.
Zentralb. f. Bakt. 1905, vol. 39, p. 285.

220. Birk. Beiträge zur Physiologie des neugeborenen Kindes.
Monats. f. Kinderh. 1911, vol. 9, p. 595.

221. Bub. Besitzt die Colostral-Milch bacterizide Eigenschaften?
Zentralb. f. Bakt. 1910, Abt. II., vol. 27, p. 321.

222. Bulloch. The transmission of hœmolysines from ascendants to descendants.
Trans. Path. Soc. 1902, vol. 53, p. 189.

223. Cattaneo. La Pediatria. 1905, p. 488. (Quoted by Frey.)

224. Conn and Stocking. Studies on the so-called germicidal action of milk.
Révue gén. du Lait. 1902-1903, vol. 2, pp. 265 and 298.

225. Conn and Esten. The comparative growth of different species of bacteria in normal milk.
Storrs' Agric. Expt. Station. 1901, p. 13.

226. Coplans, Myer. On some vital properties of milk.
Lancet. 1907, Oct. 19th.

227. Cozzolino. Uber die Vegetation von B. Coli Commune, etc.
Arch. f. Kinderh. 1902, vol. 33, p. 405.

Dehne. (*See* Hamburger.)

228. Ehrlich. Uber Immunität durch Vererbung und Säugung.
Zeit f. Hyg. 1892, vol. 12, p. 183.

229. Ehrlich and Wassermann. Uber der Gewinnung der Diphtherie-antitoxin.
Zeit. f. Hyg. 1894, vol. 18.

230. Escherich. Zur Kenntniss der Unterschiede der natürlichen und künstlichen Ernährung des Säuglings.
Wiener Klin. Wochens. 1900, vol. 13, p. 1184.

Esten. (*See* Conn.)

231. Famulener. Immunity Transmission from Mother and Offspring.
Journ. of Trop. Dis. Vol. 10, 1912, p. 332.

Findlay. (*See* Noegerath.)

Foa. (*See* Noegerath.)

232. Frey. Hœmolysiert die Frauenmilch?
Münch. Med. Wochens. 1907, vol. 52, p. 1782.

233. Friedrich. Beiträge zum Verhalten der Cholera Bakterien auf Nahrungs- und Genussm.
Arb. a.d. K. Gesunds. 1893, vol. 8, p. 465.

234. Fokker. Uber Bakterienvernichtende Eigenschaften der Milch.
Zeit. f. Hyg. 1890, vol. 9, p. 41.

235. Ganghofner and Langer. Uber die Resorption genuiner Eiweiss im Magendarmkanal neugeborenen Tiere u. Säuglinge.
Münch. Med. Wochens. 1904, vol. 34.

236. Gewin. Zur Frage des Ambozeptorgehaltes des Säuglinges Blutes.
Zeit. f. Immunitätf. 1909, vol. 1, p. 613.

Grant. (*See* van Alstyne.)

237. Gutzeit. Uber die angebliche Vermehrung der Bakterien in der Milch durch mechanische Einwirkung.
Milchw. Zentralb. 1911, vol. 7, p. 193.

238. Halban. Agglutinationsversuche mit mütterlichen und kindlichen Blute.
Wiener. Klin. Wochens. 1900.

239. Halban and Landsteiner. Uber Unterschiede des fötalen und mütterlichen Blutserums, etc.
Münch. Med. Wochens. 1902, p. 475.

240. Hamburger and Sperk. Biologische Untersuchungen über Eiweiss-Resorption vom Darm aus.
Wiener. Klin. Wochens. 1904, p. 641.

241. Hamburger. Uber Eiweissresopption bei der Ernährung.
Jahrb. f. Kinderh. 1907, vol. 65, p. 15.

242. Hamburger. Uber Antitoxin und Eiweiss.
Münch. Med.Wochens. 1907, vol. 65, No. 6.

243. Heim. Uber das Verhalten der Krankheits-Erreger der Cholera . . in Milch.
Arb. a.d. k. Gesundh. 1899, vol. 5, p. 294.

244. Hesse. Bezichungen zwischen Milch und Cholerabazillen.
Zeit. f. Hyg. 1894, vol. 17, p. 238.

245. Honigmann. Bakteriologische Untersuchungen über Frauenmilch.
Zeit. f. Hyg. 1893, vol. 14, p. 207.

246. Kaumheimer. Uber den Complementgehalt des Blutserums kranken Säuglinge.
Zentralb. f. Bakt. 1909, vol. 49, p. 208.

King. (*See* McKlintock.)

247. Kleinschmidt. Die Bakteriozidine in Frauen und Kuhmilch.
Monats f. Kinderh. 1911, vol. 10, p. 254.

248. Klimmer. Besitzt die unerhitzter Milch baktericide Eigenschaften?
Arch. f. Kinderh. 1903, vol. 36, p. 1.

249. Koch. Uber Komplementbestimmungen des kindlichen Serums.
Arch. f. Kinderh. 1909, vol. 50, p. 384.

Kolff. (*See* Noegerrat.)

250. Kolle. Milchhygienische Untersuchungen.
Klin. Jahrb. 1905, p. 319.

251. Kopf. Haptine in Rinderserum und in der Rindermilch.
Zeit. f. Hyg. 1909, vol. 63, p. 291.

252. Koning. Biologische und Biochemische Studien über die Milch.
Milchw. Zentralb. 1905, vol. 1, pp. 49, 97, 215, 289, 337.

253. Kitasato. Das Verhalten des Cholera bakterien in der Milch.
Zeit. f. Hyg. 1889, vol. 5, p. 492.

254. Kraus. Uber das Vorkommen der Immunhämagglutinine und Immun-Hämolysine in der Milch.
Wiener. Klin. Wochens. 1901, p. 737.

Landsteiner. (*See* Halban.)

255. Lane-Claypon. Report to the L.G.B. upon the available data upon the value of boiled milk, etc.
New Series No. 63.

256. Lane-Claypon. On the Presence of Hœmolytic Factors in Milk.
Journ. of Path. 1908.

Langer. (*See* Ganghofner.)

257. Langer. Zur Resorption des Colostrums.
Gesell. f. Kinderh. Dresden. 1907.

McCoy. (*See* Rosenau.)

258. McClintock and King. The oral administration of antitoxin.
Journ. of Inf. Diseases. 1906, vol. 3, p. 701, and 1909, vol. 6, p. 46.

259. Mayerhofen and Pribram. Zur Frage der Durchlässigkeit der Darmwand etc.
Zeit. f. exp. Path. and Ther. 1910, vol. 7, p. 247.

260. Michaelis and Oppenheimer. Uber die Immunität gegen Eiweisskörper.
Arch. f. Anat. und Phys. 1902, suppl., heft.

261. Moro. Biologische Beziehungen zwischen Milch und Serum.
Wiener Klin. Wochens. 1901, p. 1073; and Arch. f. Kinderh. 1902, vol. 33, p. 435.

262. Moro. Kuhmilch Präzipitin in Blute einer 4½ monat alter Atrophikers.
Münch. Med. Wochens. 1906, vol. 53, p. 214.

263. Moro. Weitere Untersuchungen über Kuhmilchpräzipitine in Säuglingsblute.
Münch. Med. Wochens. 1906, vol. 63, p. 2383.

264. Moro. Uber das bakteriolytische Alexine der Milch.
Zeit. f. exp. Path. u. Ther. 1907, vol. 4, p. 470.

265. Moro and Potpeschnig. Uber das Verhalten der Serumkomplement bei akuten Infektionskrankheiten.
Wiener. Med. Wochens. 1908, Nos. 1-3.

266. Moro. Untersuchungen über die Alexine der Milch und des kindlichen Blutserums.
Jahrb. Kinderh. 1902, vol. 53.

Moro. (*See* Schlossmann.)

Moro. (*See* Pfaundler.)

267. Much and Romer. Antitoxine und Eiweiss.
Jahrb. f. Kinderh. 1907, vol. 63.

268. Much and Happich. Uber die Antitoxische Funktion und Eiweiss.
Münch. Med. Wochens. 1907, vol. 52, p. 2589.

269. Much. Uber bakteriezidine in Perhydridasemilch.
Münch. Med. Wochens. 1908, vol. 55, I., p. 384.

270. Noegerath. Serologische Untersuchungen zur Theorie der Säuglingsernärhung. Gesell. f. Kinderh. Salzburg, 1909.
Deut. Med. Wochens. 1909, p. 1872.

271. Noeggerath, Findlay, and Foa. Ist der hämolytische Komplementgehalt des Blutserums ein Maas der Säuglings-Konstitution?
Jahrb. f. Kinderh. 1909, vol. 70, p. 732.

272. Noeggerath and Koiff. Uber die Komplement der Frauenmilch.
Jahrb. f. Kinderh. 1909, vol. 70, p. 701.

273. Noeggerath. Zur Kuhmilchhoëmolyse.
Münch. Med. Wochens. 1909, No. 48.

Obermeyer. (*See* Pick.)

Pennington. (*See* St. John.)

274. Pfaundler and Moro. Uber Hämolytische Substanzen der Milch.
Zeit. f. exp. Path. u. Ther. 1907, vol. 4, p. 551.

275. Pfaundler and Moro. Zur Physiologie und Pathologie der Säuglingsernährung.
Münch Med. Wochens. 1907, vol. 53, p. 2170.

276. Pfaundler and Moro. Uber hoëmolytisches Komplement in der Frauenmilch.
Münch Med. Wochens. 1908, No. 20.

277. Pfaundler. Experimentell Biologisches zur Frage der Säuglingsernährung.
Gesell. f. Kinderh. Koln. 1908.

278. Pfaundler. Die Antikörper Ubertragung von Mutter auf Kind.
Arch. f. Kinderh. 1908, vol. 4, p. 260.

279. Picy and Obetmeyer. Biologische-chemisch Studien über das Eiklar.
Wiener. Klin. Rund. 1902, No. 15.

Pribram. (*See* Mayerhofen.)

280. Römer. Untersuchungen über Antitoxine-Ubertragung, etc.
Berl. Klin. Wochens. 1901, vol. 38, No. 46, p. 1050.

Römer and Much. (*See* Much.)

281. Römer. Uber die Intestinale Resorption von Serumantitoxin und Milch antitoxin.
Zeit. f. Immunitätf. 1909, Orig. I., No. 2, p. 171.

282. Römer and Sames. Uber die Haltbarkeit heterologen Antitoxine in Organismus.
Zeit. f. Immunitätf. 1910, abt. I., Orig. 4.

283. Rosenau and McCoy. The germicidal properties of milk.
Journ. of Med. Research. March, 1908.

284. Rostocki. Uber den Wertt der Präzipitine, etc.
Münch. Med. Wochens. 1902, No. 18.

285. Rullmann and Trommsdorff. Milchhygienische Untersuchungen.
Arch. f. Hyg. 1906, vol. 59, p. 224.

286. Sacconaghi. Uber die Präzipitine der Verdauungsprodukte.
Zeit f. Klin. Med. 1904, vol. 51, p. 189.

287. St. John and Pennington. The relative rate of growth of milk bacteria in raw and pasteurised clean milk.
Journ. of Inf. Diseases. 1907. Nov.

288. Salge. Uber den Durchtritt des Antitoxins durch die Darmwand des menschlichen Säuglinge.
Jahrb. Kinderh. 1904, vol. 60, p. 1.

289. Salge. Immunisierung durch Milch.
Jahrb. f. Kinderh. 1905, vol. 61, p. 486.

Sames. (*See* Romer and Sames.)

Sassenhagen. (*See* Bauer.)

290. Sassenhagen. Uber die biologischen Eigenschaften der Kolostral- und Mastitis-Milch.
Arch. f. Kinderh. 1910, vol. 53.

291. Schottelius. Uber das Wachsthum der Diphtherie-bacillen in Milch.
Centralb. f. Bakt. 1896, vol. 20, p. 897.

292. Schlossmann and Moro. Zur Kenntniss der Arteigenheit der verscheidene Eiweisskörper der Milch.
Münch. Med. Wochens. 1903, vol. 14, p. 597.

293. P. Sommerfeld. Besitzen die löslichen Eiweisskörper der Milch spezifische baktericide Eigenschaften?
Centralb. f. Bakt. 1904, vol. 337, orig., p. 716.

Sperk. (*See* Hamburger.)

Stocking. (*See* Conn.)

Trommsdorff. (*See* Rullmann.)

294. Uffelmann. Beiträge zur Biologie des Cholerabacillus.
Berl. Klin. Wochens. 1892, vol. 29, p. 1209.

295. Uhlenhuth. Neuer Beitrag zum specifischen Nachwies von Eiweiss auf biologischem Wege.
Deut. Med. Wochens. 1900, No. 46.

296. Vaillard. Hérédité de l'immunité acquise.
Annales de l'Institut Pasteur. 1896, vol. 10, p. 65.

Wassermann. (*See* Ehrlich.)

297. Weigmann. Uber das Verhalten von Cholerabacillen in Milch.
Milchztg. 1894, No. 31, p. 491.

298. Wolffhugel and Redel. Die Vermehrung der Bakterie in Wasser.
Arb. a.d. K. Gesundh. 1886, vol. 1, p. 470.

INDEX.